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CASRN 90-43-7, [1,1'-BIPHENYL]-2-OL[1,1'-BIPHENYL]-2-OL (2-PHENYLPHENOL)
PUBLICLY AVAILABLE INFORMATION TO ADDRESS EPA HPV CHALLENGE RED CASE 2575

Dow is providing publicly available hazard information for the endpoints identified in the EPA HPV Challenge for orthophenylphenol. As indicated to the Agency in previous communications, this substance is a pesticide regulated by FIFRA in the U.S. and is used exclusively as an intermediate to another pesticide. Dow believes these conditions are sufficient to exempt this substance from the EPA HPV Challenge. Nearly five years after our request for exemption of 2-phenylphenol from the EPA HPV Challenge, the Agency notified Dow our request was not accepted. November 11, 2004, Dow sent a letter to EPA stating its commitment to provide publicly available information on this substance for the endpoints within the EPA HPV Challenge.

The EPA Reregistration Eligibility Decision (RED) document (Case 2575) will publish in September, 2005 on the EPA website. This document will contain physical property, biodegradation and environmental toxicity study results.

In addition, two significant publications are available for 2-phenylphenol:

1. World Health Organization Joint Meeting on Pesticide Residues, 1999, 2-Phenylphenol and Its Sodium Salt.

   This publication evaluates these pesticide substances for acceptable daily intake with a summary and review of the results of the numerous toxicological studies on 2-phenylphenol. These include acute oral, inhalation and dermal results; two guinea pig sensitization studies; dermal and eye irritation studies; palatability and toxicity in beagle dogs; 2-year dietary feeding study in mice; 91-week dietary feeding study in rats; 1-year dietary feeding study in rats; numerous genotoxicity study results including six in-vivo studies. A copy of this publication is included with this letter.

2. Bomhard, E.M., Brendler-Schwaab, S.Y.,Freyberger, A. , Herbold, B.A., K. H. Leser, K. H., Richter, M. O-Phenylphenol and Its Sodium and Potassium Salts: A Toxicological Assessment, *Critical Review in Toxicology* **32**(6):551-626, 2002.

   This article includes a review of experience in humans, teratogenicity, 2-generation reproductive study results; endocrine effects, immunotoxicology in addition to the studies described in the 1999 World Health Organization JMPR meeting report.

Dow believes this communication satisfies the commitment to provide publicly available information for all endpoints within the EPA HPV Challenge. Please remove this substance from the EPA HPV Orphans List and from Annex A of the 55th ITC Report.

Sincerely,

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cc: John Walker, USEPA Headquarters, Washington, DC

2-PHENYLPHENOL AND ITS SODIUM SALT

First draft prepared
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Explanation

2-Phenylphenol and its sodium salt were evaluated by the 1969, 1983, 1985, 1989, and 1990 Joint Meetings (Annex 1, references *12*, *40*, *44*, *56*, and *59*). A temporary ADI of 0–0.02 mg/kg bw was allocated in 1983, which was extended in 1985 and 1989. An ADI of 0–0.02 mg/kg bw was established in 1990. Since that Meeting, studies have become available on biochemical aspects, biotransformation, effects on enzymes and other biochemical parameters, acute toxicity, short-term toxicity, long-term toxicity, genotoxicity, reproductive toxicity, dermal and ocular irritation and dermal sensitization, and on the mechanism of the carcinogenic effect in the rat urinary bladder. The compound was reviewed by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues.

The toxicological data on the sodium salt of 2-phenylphenol were not used to establish the ADI, since the salt rapidly dissociates to 2-phenylphenol. These data were, however, considered of value for the review and are therefore included.

Evaluation for Acceptable Daily Intake

1. Biochemical aspects

(a) Absorption, distribution, and excretion

Groups of four male Fischer 344 rats were given single oral doses of [^{14}C]2-phenylphenol (purity, 99.8%; specific activity, 19 mCi/mmol) or [^{14}C]sodium 2-phenylphenol (purity, 98.7%) by gavage at a dose of 500 mg/kg bw and were immediately placed in glass metabolism cages.

About 90–95% of the administered radiolabel on both compounds was recovered in urine and 5–6% in faeces, mainly during the first 24 h. The rates of urinary excretion were virtually identical in the two groups. In a second experiment, animals were fed diets containing 13 000 ppm of 2-phenylphenol or 20 000 ppm of the sodium salt (equimolar amounts) for 2 weeks before administration of single oral doses of the labelled compounds. The animals still eliminated most of the radiolabel (88–94%) in urine and small amounts in faeces (3–5%). Preconditioning did not greatly affect the disposition of radiolabel, although the sodium salt appeared to have been eliminated somewhat more rapidly than 2-phenylphenol (Reitz et al., 1983).

Groups of four male Fischer 344 rats were given single oral doses of [^{14}C]2-phenylphenol (purity not given; specific activity, 1.6 mCi/mmol) at 160 mg/kg bw or [^{14}C]sodium 2-phenylphenol at 250 mg/kg bw (equimolar levels; purity not given; specific activity, 1.6 mCi/mmol). The animals were fasted overnight before and for 6 h after dosing. Urine and faeces were collected daily for 7 days. The excretion patterns in the two groups did not differ significantly, and 82–98% of the dose was recovered in urine and only 2–5% in faeces within 24 h of dosing. Two male rats received bile duct cannulae, and bile was collected for 3 days after a single oral dose of 250 mg/kg bw of radiolabelled sodium salt. Excretion of radiolabel in the bile began within the first hour of dosing, reached a peak within 3–6 h, and was almost complete by 8 h' about one-fourth of the dose was recovered in bile over the 3-day collection period. The authors interpreted these results as indicating rapid absorption from the intestine and enterohepatic circulation of 2-phenylphenol metabolites. The pattern of distribution in organs and tissues, examined on days 1, 3, and 7 after administration of the sodium salt and on days 1 and 7 after administration of 2-phenylphenol, showed little difference. Little radiolabel was retained in organs and tissues. including the urinary bladder (Yamaha et al., 1983; Sato et al., 1988).

In a comparative study, [^{12}C/^{13}C/^{14}C]2-phenylphenol (purity, 99.5%; specific activity, 48 mCi/mmol) was given to 10 male B6C3F$_1$ mice as a single oral dose of 15 or 800 mg/kg bw, to 10 male and 10 female Fischer 344 rats as a single oral dose of 28 or 27 mg/kg bw, and to six male volunteers as a dermal dose of approximately 6 µg/kg bw on the forearm for 8 h. The compound was well absorbed in the mice, 84% and 98% of the two doses being recovered in urine collected over 48 h. Extensive absorption and rapid elimination were also seen in the rats, 89 and 86% of the dose being found in the urine of males and females, respectively, within 24 h. 2-Phenylphenol was also rapidly eliminated by the volunteers, 99% of the absorbed dose being collected in urine within the first 48 h of exposure (Bartels et al., 1998).

The skin of the forearm of six male volunteers aged 19–27 and weighing 58–98 kg was exposed to 100 µl of a 0.4% isopropanol solution of [^{13}C/^{14}C]2-phenylphenol providing a dose of approximately 6 µg/kg bw and 42 µCi for 8 h. Samples of blood, urine, and faeces were collected at various times for five days, and blood samples were also taken during exposure. High concentrations of radiolabel in blood were observed within the first 2 h of the start of exposure in all subjects, indicating rapid absorption. The rate diminished fairly rapidly at the end of the exposure period, and little or no radiolabel was present in venous blood samples collected 2 days after termination of exposure. About 43% of the applied dose of 2-phenylphenol was absorbed, about 58% of which was recovered in swabs, skin rinse, gauze, and the protective enclosure. The majority (99%) of the absorbed compound was excreted in urine, and faeces represented a minor route of elimination (1% within 5 days). A mean of 0.04% of the administered radiolabel was found in the tape strips covering the application site, indicating no accumulation in the superficial layers of the skin (Selim, 1996).

The plasma concentrations peaked within 4 h of dosing and then declined rapidly, virtually all of the absorbed dose being excreted in urine within 24 h. A one-compartment model was used to describe the pharmacokinetics of absorption and clearance of [^{14}C]2phenylphenol in these volunteers. Approximately 43% of the applied dose was absorbed through the skin, with an average absorption half-time of 10 h. Once absorbed, its renal clearance was rapid, with an average elimination half-time of 0.8 h. Overall, the pharmacokinetics of [^{14}C]2-phenylphenol was similar in the individual volunteers, and the model parameters were in excellent agreement with the experimental data. The rapid excretion in urine indicates that 2-phenylphenol is unlikely to accumulate in humans exposed repeatedly (Timchalk, 1996).

(b) Biotransformation

Groups of 10 male B6C3F$_1$ mice were given a single oral dose of [^{14}C]2-phenylphenol (specific activity, 48 mCi/mmol) at 25 or 1000 mg/kg bw or five daily doses of unlabelled compound (purity, 99.5%) at 1000 mg/kg bw per day followed by a single oral dose of labelled compound at 1000 mg/kg bw and were killed 48 h after dosing. For comparison, groups of two male and two female Fischer 344 rats were given a single oral dose of labelled compound at 25 or 125 mg/kg bw and were killed 24 h after dosing. The excretion of [^{14}C]2-phenylphenol in mice was rapid and was complete by 12–24 h after dosing, with 74–98% of the recovered radiolabel in urine and 6–13% in faeces; < 1% was recovered in the tissues and carcass. Eight radiolabelled metabolites were detected in the urine of both mice and rats, with no major differences in distribution by species, by sex in the rats, or single or repeated dosing in mice. A small amount (0.4%) of free 2-phenylphenol was detected only in urine of female rats given the single dose of 125 mg/kg bw. Four major urinary metabolites were identified: phenylhydroquinone glucuronide, phenylhydroquinone sulfate, 2-phenylphenol sulfate, and 2-phenylphenol glucuronide, accounting for about 98% of the recovered dose in mice and 102% in rats. An additional metabolite which accounted for about 2.7% of the recovered dose in rat urine was tentatively identified as the sulfate conjugate of 2,4'-dihydroxybiphenyl. No qualitative difference in metabolites was observed in male mice, but a dose-dependent, quantitative difference was noted in the extent of sulfation and glucuronidation of 2-phenylphenol. After a single dose of 25 mg/kg bw to mice, the sulfate was the major urinary metabolite, accounting for 56% of the recovered radiolabel, while the glucuronide accounted for 29%. After single or repeated doses of 1000 mgkg bw, the glucuronide was the major metabolite, accounting for 48–60% of the urinary radiolabel, while the sulfate accounted for 20–27%. In rats given a single oral dose of 25 mg/kg bw, 2-phenylphenol sulfate was the major metabolite, accounting for 91% of the recovered radiolabel, while the glucuronide accounted for only 7%. Formation of phenylhydroquinone glucuronide and sulfate represented minor metabolic pathways, accounting for 11–23% and 2–7% of the radiolabel in mice and rats, respectively. The extent of conjugation was not dose-dependent in mice given a single oral dose of 25 or 1000 mg/kg bw of 2-phenylphenol. The authors concluded that 2phenylphenol is completely metabolized in mice and rapidly eliminated in the urine, predominantly as the sulfate and glucuronide conjugates. The extent of metabolism was qualitatively comparable in mice and rats, although quantitative differences were seen in the extent of conjugation (McNett et al., 1997).

In the comparative study of Bartels et al. (1998) described above, sulfation of 2-phenylphenol was found to be the major metabolic pathway at low doses in all three species, accounting for 57% of the urinary radiolabel in male mice given 15 mg/kg bw, 82% in male rats given 28 mg/kg bw, and 69% in the male volunteers given 0.006 mg/kg bw. The glucuronide was also found, representing 29, 7, and 4% of the total urinary metabolites at these low doses in the three species, respectively. Conjugates of phenylhydroquinone accounted for 12, 5, and 15% of the dose in mice, rats, and humans, respectively. Little or no free 2-phenylphenol was found in any species, and no free phenylhydroquinone or phenylbenzoquinone was found in any species, with a limit of detection of 0.1–0.6%. A novel metabolite, the sulfate conjugate of 2,4'-dihydroxybiphenyl, was identified in rats and humans, comprising 3 and 13% of the low doses, respectively. Dose-dependent shifts in the conjugation of parent 2-phenylphenol were seen in mice, indicating saturation of the sulfation pathway after the high dose of 800 mg/kg bw. Dose-dependent increases in the total amount of phenylhydroquinone were also observed in the mice.

The major metabolites identified in the urine of five male and five female Fischer 344 rats fed 20 000 ppm of sodium 2-phenylphenol (purity not given) in the diet for 136 days were glucuronide conjugates of 2-phenylphenol and 2,5-dihydroxybiphenyl. Trace amounts of 2,5-diquinonebiphenyl were also tentatively identified. Unconjugated phenolic metabolites accounted for only 1% of the phenolic metabolites excreted; no other metabolites were found. By 24 h after feeding, 55% of the dose had been recovered in males and 40% in females. A sex difference was found in the proportion of urinary metabolites, male rats excreting 1.8 times as much conjugated 2-phenylphenol and more than 7 times as much conjugated 2,5-dihydroxybiphenyl as female rats in 24-h urine samples. No explanation was given for the inability to find the sulfate ester of 2-phenylphenol in urine in this study. As only 40–55% of the administered dose was recovered, it may have been present but not identified (Nakao et al., 1983).

Single oral doses of 5, 50, or 500 mg/kg bw of [^{14}C]2-phenylphenol (purity, 99.8%; specific activity, 19 mCi/mmol per L) or [^{14}C]sodium 2-phenylphenol (purity, 98.7%; specific activity, 19 mCi/mmol/L) were administered to groups of four male rats, and the urinary metabolites were identified and quantitified. At the two lower doses, the major metabolites of both compounds were the glucuronide and sulfate ester conjugates of 2-phenylphenol, and unconjugated 2-phenylphenol and 2,5-dihydroxybiphenyl accounted for < 2% of the total radiolabel recovered in urine at a limit of detection of 1–2%. Nearly identical high-performance liquid chromatograms were obtained for the two compounds. At 500 mg/kg bw, a further metabolite of both compounds was identified, which accounted for 20–30% of the urinary radiolabel and appeared to be a conjugated dihydroxybiphenyl molecule, most likely with glucuronide and/or sulfate groups. The authors hypothesized that this metabolite is formed only at high doses as a result of saturation of normal glucuronide and sulfate ester conjugation pathways. Incubation of [^{14}C]2-phenylphenol with purified microsomes *in vitro* in the absence of conjugating substrates yielded large amounts of a material which co-chromatographed with 2,5-dihydroxybiphenyl. The semiquinone and quinone were not identified in these studies, but their formation was proposed on the basis of the results of similar studies on benzene (Reitz et al., 1983).

In a study of toxicity in male Fischer 344 rats given diets containing 0, 800, 4000, 8000, or 12 500 ppm of 2-phenylphenol (purity, 99.5%) for 13 weeks, the DNA of the urothelium was isolated at the end of the study and examined for covalent adducts of 2-phenylphenol by the ^{32}P-postlabelling assay. The concentrations of 2-phenylphenol metabolites were also measured in overnight urine samples collected from the animals at the end of the study. The glucuronide and sulfate conjugates of 2-phenylphenol and the hydroxylated metabolite, 2,5-phenylhydroquinone, were found to be the major metabolites. The major conjugation in all samples was with sulfate. The formation of this metabolite appeared to be saturated at 8000 ppm, while the concentrations of the remaining three conjugated metabolites increased in a dose-dependent fashion up to the high dose. Traces of free 2-phenylphenol and phenylhydroquinone were observed at all doses, free phenylhydroquinone comprising 0.6–1.5% of the total metabolites measured. The concentrations of creatinine were comparable in all groups (Bartels & McNett, 1996).

Mature cats and dogs were given [^{14}C]sodium 2-phenylphenol (purity and specific activity not given) at single oral doses ≤ 3 g/kg bw. The amount of radiolabel in plasma was higher in dogs than in cats, and the dogs metabolized and excreted three times more radiolabel in urine than cats. The urinary metabolites were unchanged 2-phenylphenol, glucuronide and sulfate conjugates, and phenol derived from cleavage of the phenylphenol bond and ring hydroxylation. The phenol metabolites were derived from both 2-phenylphenol ring moieties (Oehme & Smith, 1972).

Urine samples were collected weekly after single oral doses every second day of [^{14}C]2-phenylphenol (purity, 95%) to three male and three female mature beagle dogs (0.3 mg/kg bw per day), three male and three female immature beagle dogs (2.0 mg/kg bw per day), three male and three female mature domestic cats (1.2 mg/kg bw per day), and three male and three female immature domestic cats (2.0 mg/kg bw per day) for 8 weeks. The main urinary excretion product was unchanged 2-phenylphenol, representing 70–90% of the radiolabel in dogs and 95–98% in cats. Dogs excreted significantly more glucuronide- and sulfate ester-conjugated 2-phenylphenol than cats, and immature dogs excreted four times as much glucuronide conjugate as mature dogs. The age differences did not affect the rate of excretion of the sulfate ester conjugate in either species (Savides & Oehme, 1980).

In the study of Selim (1996) in volunteers treated dermally, described above, 99% of the absorbed dose of 2-phenylphenol was eliminated in urine, primarily as polar conjugates or hydroxylated metabolites. The major urinary metabolite was the sulfate conjugate, which accounted for 68% of the absorbed dose; conjugation with glucuronic acid accounted for only 3%. Hydroxylation of the phenol or phenyl ring, followed by conjugation, was also significant, phenylhydroquinone glucuronide representing 14% of the absorbed dose and 2,4'-dihydroxybiphenyl sulfate, 12%. Traces of unmetabolized parent compound (0.5% of absorbed

dose) were found only in samples taken shortly after administration. No free phenylhydroquinone or phenylhydroquinone-sulfate was found in urine (Bartels et al., 1997; Timchalk et al., 1998).

The proposed metabolic pathways of 2-phenylphenol in rodents and humans are shown in Figure 1.

(c) Effects on enzymes and other biochemical parameters

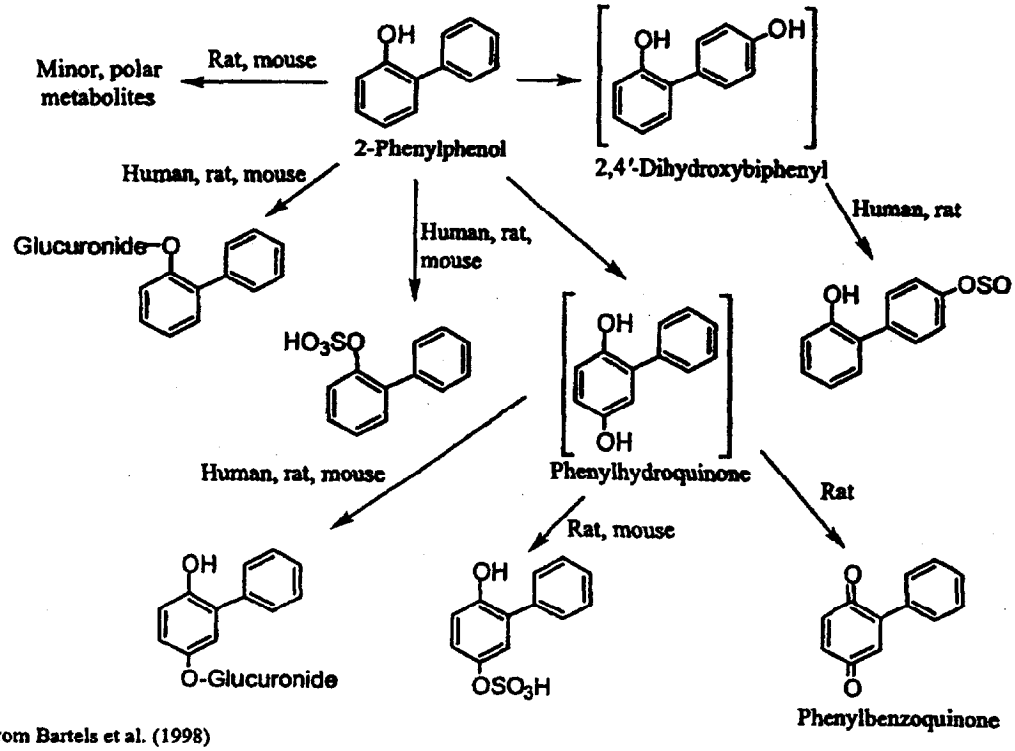
2-Phenylphenol

2-Phenylphenol was converted to 2,5-dihydroxy biphenyl (phenylhydroquinone) by microsomal cytochrome P450 enzymes. Depending on the cofactor used, the microsomal enzymes catalysed either oxidation and/or reduction of the metabolite. Phenylhydroquinone was oxidized to phenyl 2,5'-*para*-quinone by cumene hydroperoxide-supported enzymes, and this compound was reduced to phenylhydroquinone by cytochrome P450 reductase. This study provides direct evidence of cytochrome P450-catalysed redox cycling of 2phenylphenol, which may play a role in the induction of bladder cancer by this substance (Roy, 1990).

Activation of the 2-phenylphenol metabolite phenylhydroquinone by prostaglandin (H) synthase in the presence of arachidonic acid and hydrogen peroxide was studied to test the hypothesis that prostaglandin synthase in rat urinary bladder transitional epithelium and kidney medullar papilla is responsible for activation of the metabolite to reactive intermediates in the bladder and kidney. Phenylhydroquinone was found to be metabolized by the peroxidase activity of prostaglandin synthase and by other peroxidases such as horseradish peroxidase and myeloperoxidase, suggesting that the peroxidative metabolism of phenylhydroquinone could play a role in urinary bladder and kidney carcinogenesis in rats (Kolachana et al., 1991).

In a study of the effect of pH on nonenzymatic oxidation of phenylhydroquinone, the effects of phenylbenzoquinone and oxygen concentration on autoxidation of phenylhydroquinone, and

Figure 1. Proposed pathways of metabolism of 2-phenylphenol in mice, rats, and humans



From Bartels et al. (1998)

the nonenzymatic conversion of phenylbenzoquinone to phenylhydroquinone, a curvilinear relationship was found between the rate of oxidation of phenylhydroquinone and pH over the range 6.3–7.6. Phenylbenzoquinone was formed during autoxidation of phenylhydroquinone, with a yield of 0.92 ± 0.02. The results indicate that the production of reactive metabolites from phenylhydroquinone involves both a pH-independent (i.e. oxygen-dependent) and a pH-dependent pathway and that the presence of phenylbenzoquinone enhances the rate of pH-dependent phenylhydroquinone autoxidation. The authors suggested that ionization of phenylhydroquinone semiquinone is a key step in production of reactive species in the pH-dependent pathway. They found a good correlation between the proposed reaction pathway and the induction by 2-phenylphenol of bladder lesions in rats. Thus, pH-dependent autoxidation of free phenylhydroquinone in urine may be responsible for the tumorigenic effects of 2-phenylphenol and sodium 2-phenylphenol in the rat bladder (Kwok & Eastmond, 1997).

Groups of eight female B6C3F$_1$ mice were given 0, 1, 10, or 200 mg/kg bw per day of 2-phenylphenol (purity, > 98%) by gavage on 5 days per week for 2 weeks. As a positive control, mice were given 45 mg/kg bw of cyclophosphamide intraperitoneally for 4 days. The weights of the body, liver, spleen, kidney, and thymus were recorded, and samples were prepared for histopathological examination. Haematology and clinical chemistry were conducted, and bone-marrow cellularity and colony formation, lymphoproliferative responses, delayed hypersensitivity responses, immunoglobulin, antibodies, response to *Listeria monocytogenes* challenge, and tumour susceptibility were studied. None of the treated animals died or showed signs of toxicity. Histopathological examination revealed no significant lesion in any tissues. The weight of the thymus and the relative weight of the spleen were slightly increased at 200 mg/kg bw per day. The slight haematological alterations seen did not show a dose–response relationship and were within the normal range of biological variation. A slight increase in serum cholesterol concentration and a corresponding decrease in triglyceride concentration were seen in mice at 200 mg/kg bw per day. The activity of alanine aminotransferase and total protein in serum were not affected, although the albumin:globulin ratio was slightly decreased at the high dose. Bone-marrow cellularity, lymphoproliferative responses, immune function, and host susceptibility were not altered. In contrast, treatment with cyclophosphamide resulted in marked alterations. The authors concluded that 2-phenylphenol, even at relatively high doses, did not alter immune function or host susceptibility (Luster et al., 1981).

Sodium 2-phenylphenol

Binding of sodium 2-phenylphenol metabolites to macromolecules *in vitro* was studied by incubating [^{14}C]sodium 2-phenylphenol (specific activity, 19 mCi/mmol) with purified liver microsomes from male rats in the presence of a NADPH regenerating system and bovine serum albumin, which served as a 'protein acceptor'. Macromolecular binding of radiolabel to protein, which was dependent on the presence of both active microsomes and NADP, was observed. In order to study the binding of metabolites of 2-phenylphenol and its sodium salt to macromolecules in the liver, kidney, and urinary bladder *in vivo*, groups of four male rats were given single oral doses of ^{14}C-labelled compounds at doses of 50, 100, 200, or 500 mg/kg bw, and tissues were excised 16–18 h later for measurement of macromolecular binding, which was determined as nanomoles of bound material per milligram of protein. The extent of binding was not linearly related to the administered dose. Disproportionate increases were seen in each tissue at doses of sodium 2-phenylphenol ≥ 200 mg/kg bw and in liver and bladder at doses of 2-phenylphenol at 200–500 mg/kg bw (Reitz et al., 1984).

Sodium 2-phenylphenol (purity not given) was administered in the diet at a concentration of 20 000 ppm to 4-week-old male and female Fischer 344 rats for 136 days. Urine was collected periodically. At the end of treatment, the rats were killed, blood samples were collected, and livers and kidneys were removed. The amounts of cyclic nucleotides (cAMP and c-GMP) were determined in urine, plasma, liver, and kidneys, and adenylate cyclase activity was measured in liver and kidneys. In male rats, the c-AMP levels in urine and plasma were decreased whereas the c-GMP levels were increased. In females, c-AMP levels were decreased only during the first 3 days of feeding, and the levels of c-AMP and cGMP in liver and kidneys were unchanged. The

decreased urinary c-AMP in male rats was probably the result of decreased adenylate cyclase activity in liver and kidneys. A similar change in adenylate cyclase activity was observed in liver but not in kidneys of female rats treated with sodium 2-phenylphenol. The sex-related alterations in cyclic nucleotide levels were postulated to be involved in the sex-dependent induction of urinary bladder tumours by sodium 2-phenylphenol (Nakagawa et al., 1984).

In male and female Fischer 344 Du Crj rats given 20 000 ppm of sodium 2-phenylphenol in the diet for 20 weeks, urinary γ-glutamyl transpeptidase activity decreased immediately after the start of treatment and remained low throughout the study. The activities of this enzyme and of alkaline phosphatase in kidney homogenate were found to have decreased to about 80% of the control values at 20 weeks, but the activity of glucose-6phosphate dehydrogenase was significantly increased; that of Na/K-ATPase was unchanged. In liver homogenate, however, γ-glutamyl transpeptidase activity was increased by about eight times and that of glucose-6-phosphate dehydrogenase was significantly increased, but the activities of alkaline phosphatase and Na/K-ATPase were not significantly different from the control values. The glutathione concentration in the livers of treated rats was significantly reduced (Nagai & Nakao, 1984).

2. Toxicological studies

(a) Acute toxicity

The results of studies of the acute toxicity of 2-phenylphenol and its sodium salt are summarized in Table 1. The clinical signs of toxicity were generally nonspecific.

(b) Short-term studies of toxicity

2-Phenylphenol

Rats

2-Phenylphenol (purity, 99.8%) was administered to 30 male Fischer 344 rats (Charles River) in the diet at a concentration of 20 000 ppm, equal to 1000 mg/kg bw per day, for up to 90 days. Interim sacrifices were performed at 3, 7, 30, and 65 days. Only seven rats at each dose were permitted to live to 90 days, at which time they were killed. Food consumption and body weight were markedly reduced within the first week and remained low throughout the study. The renal lesions observed in these rats included focal cortical cysts, significantly decreased urine specific gravity (at 65 and 90 days), small amounts of blood in the urine, focal tubular collapse and atrophy in the cortex, and cystic degeneration (at 65 and 90 days). No treatment-related urinary bladder lesions were observed. A NOAEL could not be identified since the body weight was reduced at the only dose tested (Reitz *et al.*, 1983).

Table 1. Acute toxicity of 2-phenylphenol and its sodium salt

Species	Sex	Route	LD_{50} (mg/kg bw) or LC_{50} (mg/L)	Reference
2-Phenylphenol				
Mouse	M	Oral	1200	Taniguchi et al. (1981)
	F		1100	
Mouse	M	Oral	3500	Tayama et al. (1983, 1984)
	F		3200	
Rat	M	Oral	2600	Tayama et al. (1980)
	F		2900	
Rat	M	Oral	2800	Gilbert & Crissman (1994)
	F		2800	
Rat	M&F	Inhalation (4 h)	> 36	Landry et al. (1992)
Rabbit	M&F	Dermal	> 5000	Carreon & New (1981)
Sodium 2-phenylphenol				
Mouse	M	Oral	900	Ogata et al. (1979)
	F		800	
Rat	M	Oral	1700	Taniguchi et al. (1981)
	F		1600	
Rat	M	Oral	1100	Tayama et al. (1979)
	F		1100	
Rat	M	Oral	850	Gilbert & Stebbins (1994)
	F		590	

Groups of 10 male and 10 female Fischer 344 rats were fed diets containing 0, 1300, 3100, 6300, 13 000, or 25 000 ppm of 2-phenylphenol (purity not given), equal to 0, 180, 390, 760, 1700, and 2800 mg/kg bw per day for males and 0, 200, 410, 800, 1700, and 3000 mg/kg bw per day for females, for 12 weeks. Body weight and body-weight gain were severely depressed in males and females at 25 000 ppm and to a lesser extent (14%) in males fed 13 000 ppm. No significant treatment-related effects were seen in analyses of urine performed at weeks 9 and 13. Haematological and blood chemical values were generally normal, other than a slight decrease in haemoglobin concentration in male and female rats at the highest dose. The absolute and relative weight of many organs in male rats at this dose were significantly decreased. The NOAEL was 6300 ppm, equal to 760 mg/kg bw per day, on the basis of reduced body weight and body-weight gain at 13 000 ppm (Iguchi *et al.*, 1984).

Groups of five male and five female Fischer 344 rats received dermal applications of 0, 100, 500, or 1000 mg/kg bw per day of 2-phenylphenol (purity, 99.8%) once daily on 5 days per week for a total of 15 applications over 21 days. The amount applied per animal was adjusted weekly on the basis of the body weight of individual animals and was applied to a 5 cm x 5 cm area of clipped skin on the back, covered with a nonabsorbent cotton patch held in place by an elastic wrap secured with adhesive tape. The wraps and patches were removed not less than 6 h after treatment, and the treated area was wiped with a wettened gauze pad to remove residual test material. Control animals were handled in the same way. All animals were acclimated to the wraps for 2 days before the beginning of treatment. They were observed at least daily and were given a complete clinical examination weekly. The skin at the site of treatment was examined after removal of the wrap on the last day of dosing, each week, and on the day before necropsy. Body weights were measured weekly and feed consumption and feed efficiency were calculated weekly. Urine was analysed on day 19. The rats were fasted overnight before necropsy, when haematological and serum clinical chemical parameters were evaluated, all animals were examined for gross pathological changes, selected organs were weighed, and tissues were preserved. Selected tissues and all gross lesions from animals in the control and high-dose groups were examined histologically.

No deaths occurred at any dose. Treatment-related effects indicative of dermal irritation were observed at the site of application in animals of each sex at 500 and 1000 mg/kg bw per day. Female rats appeared to be slightly more sensitive than males, but the severity of lesions increased with duration of exposure and dose in both sexes. The irritating effects ranged from scaling to fissures. Body weights and feed consumption were not affected, and no significant treatment-related effects were found in the haematological, clinical chemical, and urinary parameters evaluated. No treatment-associated alterations were found in the liver or kidneys (Zempel & Szabo, 1993).

Guinea-pigs

2-Phenylphenol was evaluated in 10 male Hartley albino guinea-pigs for dermal sensitization potential by a modified Buehler method. The animals received three dermal applications of 0.4 g of 2-phenylphenol (purity, 99.9%) during a 3-week induction period and were challenged with 0.4 g of the compound 2 weeks after the last induction. The condition of the test sites was assessed approximately 24 and 48 h after the challenge. No erythema or oedema was seen in any of the animals. The author concluded that 2-phenylphenol did not cause delayed contact hypersensitivity (Berdasco, 1991).

Ten male Hartley albino guinea-pigs were clipped free of hair on the day before dosing and received three dermal applications of 0.4 g of 2-phenylphenol (purity, 99.9%) moistened with 0.20 ml of distilled water during a 3-week induction period. Two weeks after the last induction application, they were given a challenge application of 0.4 ml of a 7.5% suspension of the compound in water on another site for 6 h. Five animals received no induction but a 0.4-ml aliquot of a 7.5% suspension of 2-phenylphenol in water. The condition of the test sites was assessed approximately 24 and 48 h after challenge. No erythema was seen, and none of the uninduced animals showed irritation. The animals were in good health and gained weight during the study. The author concluded that 2-phenylphenol did not cause delayed contact hypersensitivity (Gilbert, 1994b).

Rabbits

The ability of 2-phenylphenol (purity, 99.9%) to cause primary dermal irritation was studied in three male and three female New Zealand white rabbits that received applications of aliquots of 0.5 ml of the substance moistened with 0.3 ml of distilled water for 4 h on intact skin on a clipped, 10 cm x 10 cm area of the back. The application sites were graded for erythema and oedema within 30 min and 24, 48, and 72 h of removal of the patch and on days 7, 8, 9, 10,11, 14, and 15. The animals were weighed on the day of treatment and at the end of the study. Very slight erythema was observed at the application site in one of the six rabbits within 30 min of removal of the test material and in two rabbits 24 h later. Severe to slight eschar formation was observed in four rabbits within 30 min of treatment which persisted throughout the remainder of the study. Four animals had burns at the site of application within 30 min, which resolved as scabs and then scars by the end of the study. Four rabbits had very slight-to-severe oedema 30 min and 24 h after removal of the test material, and slight-to- severe oedema was observed on three animals 48 and 72 h after removal. Body weight was not affected (Gilbert, 1994a).

Instillation of 0.1 g of 2-phenylphenol (purity not given) into the right eye of six New Zealand white rabbits resulted in moderate corneal injury, iritis, and moderate-to-severe conjunctival redness and chemosis in all animals 24, 48, and 72 h and 7 days after dosing (Norris, 1971).

Dogs

In studies to assess the palatability and toxicity of 2-phenylphenol (purity, 99.8%) in beagle dogs, males and females were treated with several regimens. For palatability, one female was given feed containing 2-phenylphenol to give a dose of 300 mg/kg bw per day for 5 days. In a study to assess toxicity, groups of two males and three females were given 300–1000 mg/kg bw per day as a solution in peanut oil by gastric intubation for up to 9 days or 400–700 mg/kg bw per day by capsule for 1–2 days. In a 4-week study, groups of two males and two females were given 0, 100, 200, or 300 mg/kg bw per day as a solution in peanut oil by gastric intubation on 5 days per week. In a 1-year study, groups of four males and four females were given 2-phenylphenol as a solution in peanut oil by gastric intubation at doses of 0, 30, 100, or 300 mg/kg bw per day for 5 days per week for 1 year. The animals were examined daily for clinical signs, and body weight, feed consumption, haematological, urinary, and clinical chemical parameters, organ weights, ophthalmologic endpoints, and gross and histopathology were determined.

Administration of 300 mg/kg bw per day in feed or by intubation for 5 days resulted in decreased body weights and feed consumption that correlated with the unpalatability of 2-phenylphenol. Repeated emesis was seen at doses ≥ 400 mg/kg bw per day administered in gelatin capsules or in peanut oil by intubation and at doses ≥ 200 mg/kg bw per day by intubation throughout the 4-week study. Dose-related emesis was also seen in animals given 2-phenylphenol for 1 year. In general, more frequent emesis and ejection of greater volumes of gastric content was seen in dogs given 300 mg/kg bw per day than in those given lower doses. While emesis effectively limited the dose that could be retained, the degree of emesis did not appear to compromise the health of the animals over 1 year. The reaction was categorized as a local, transitory response of the mucosal lining of the upper alimentary tract rather than a reaction of the central nervous system. No adverse effects were seen on body weight, feed consumption, haematological, urinary, clinical chemical, or ophthalmological parameters, organ weights, or gross or histological appearance of a range of tissues from all dogs. The only deaths were of two males at the high dose in the 1-year study which died subsequent to inadvertent deposition of the test solution into the lungs after approximately 4.5 months of dosing. The NOAEL was 300 mg/kg bw per day (Cosse *et al.*, 1990).

Sodium 2-phenylphenol

Mice

Groups of 10 male and 10 female $B6C3F_1$ mice were fed diets containing 0, 2500, 5000, 10 000, 20 000, or 40 000 ppm of sodium 2-phenylphenol as the tetrahydrate (purity not given), equivalent to 0, 270, 550, 1100, 2200, and 4400 mg/kg bw per day, for 13 weeks. Body-weight gain was significantly depressed in males fed 10 000 or 20 000 ppm and in animals of each sex fed

40 000 ppm. Urinary analysis showed increased pH and decreased specific gravity at the highest dose. The relative weights of the livers of animals given 10 000, 20 000, or 40 000 ppm were significantly greater than those of controls, but no treatment-related histopathological findings were made. Light and scanning electron microscope examination of the bladder epithelium at 4, 8, and 13 weeks in three males and three females in the control group and at 20 000 ppm showed no abnormaility in the appearance of the bladder epithelium of treated mice at any time. The NOAEL was 5000 ppm, equivalent to 550 mg/kg bw per day, on the basis of reduced body-weight gain and increased relative liver weight at 10 000 ppm (Shibata et al., 1985).

Rats

Sodium 2-phenylphenol (purity, 98.7%) was administered in the diet to 30 male Fischer 344 rats at a concentration of 20 000 ppm for up to 90 days. Interim sacrifices were performed at 3, 7, 14, 30, and 65 days. Only seven rats per group were permitted to live to 90 days, at which time they were killed. The lesions seen in the urinary bladder epithelium were increased mitosis beginning at 3 days and thickening (i.e. simple hyperplasia) beginning at 14 days. No tumours were observed in the bladder. A NOAEL could not be identified since lesions were observed in the bladder at 20 000 ppm, the only dose tested (Reitz et al., 1983).

Groups of 10 male and 10 female Fischer 344 rats were fed diets containing 0, 1250, 2500, 5000, 10 000, 20 000, or 40 000 ppm of sodium 2-phenylphenol (purity, >95%), equal to 0, 85, 180, 350, 710, 1400, and 2500 for males and 0, 87, 180, 350, 690, 1300, and 2400 mg/kg bw per day for females, for 13 weeks. The rats were observed daily for changes in general condition and were weighed weekly; the amounts of feed and water consumed were measured on 3 days every other week. No deaths occurred during the study. A 15–17% decrease in body-weight gain was seen in animals at doses ≥ 5000 ppm. Urinary bladder tumours occurred in male rats at frequencies of 1/10 at 10 000 ppm, 9/10 (five transitional-cell carcinomas) at 20 000 ppm, and 1/10 at 40 000 ppm. Six rats at 40 000 ppm had pyelonephritis. In female rats, the frequencies of tumours were 0/10 at 20 000 ppm and 2/10 (papillomas only) at 40 000 ppm. No bladder calculi were observed in this experiment. The NOAEL was 2500 ppm, equal to 180 mg/kg bw per day, on the basis of reduced body-weight gain at 5000 ppm (Iguchi et al., 1979; Hiraga & Fujii, 1981).

Guinea-pigs

Sodium 2-phenylphenol (purity, 99.1%) was applied to the clipped skin of 10 male Hartley albino guinea-pigs as a 0.4-ml aliquot of a 0.5% suspension in distilled water at 3-week intervals for induction. Two weeks after the last dose, a challenge of 0.4 ml of a 0.1% solution of the compound in distilled water was applied to the other side of the animals. No erythema occurred at the test site. The animals gained weight throughout the study. The author concluded that sodium 2-phenylphenol did not cause delayed contact hypersensitivity (Gilbert, 1994c).

Rabbits

Sodium 2-phenylphenol diluted 1:200 with distilled water was instilled into the eyes of six rabbits (strain not given). Temporary, mild conjunctival reactions were observed in three animals. The authors concluded that the compound slightly irritated the eye (Davies & Liggett, 1973).

(c) Long-term studies of toxicity and carcinogenicity

2-Phenylphenol

Mice

Groups of 50 male and 50 female B6C3F$_1$ mice were fed diets (concentrations not given) supplying 2-phenylphenol (purity, 99.9%) at doses of 0, 250, 500, or 1000 mg/kg bw per day for 2 years. A satellite group of 10 male and 10 female mice at each dose was maintained on the diets for 12 months and then necropsied. All mice were observed at least once daily for overt signs of toxicity, and a thorough clinical examination was performed at least once a week throughout the study. Body weights and feed consumption were recorded weekly for the first 13 weeks and monthly thereafter. The diets were prepared weekly or every other week, with adjustments of the

2-phenylphenol concentration according to group mean body weights and feed consumption to maintain the desired doses for each group.

Clinical signs and mortality rates were unaffected by treatment, but decreased body weights (by 6–20%) and weight gain (by 10–38%) were seen in all treated groups except for males fed 250 mg/kg bw. Haematological, clinical chemical, and urinary parameters in mice necropsied at 12 and 24 months showed no consistent, toxicologically significant alterations indicative of target organ toxicity. Changes in the weights of the adrenal glands, brain, heart, kidneys, liver, testis, and spleen which were found to be statistically significant were confounded by the marked decrease in body weight. Nevertheless, the consistent increases in the absolute and/or relative weights of the liver at all doses suggests a treatment-related effect. Gross observations at necropsy in males at the high dose at 12 months and in males at the intermediate and high doses at 24 months showed a slight increase in the number of mice with liver masses or nodules. Microscopic examination of the livers of mice at 12 and 24 months revealed treatment-related effects at all doses. The cytoplasm of hepatocytes stained homogeneously, indicating liver enzyme induction, but there was no evidence of degeneration or necrosis. The microscopic changes were dose-related and resembled those associated with adaptation to metabolic demands. An increased incidence of eosinophilic hepatocellular foci was also observed in males at 100 and 500 mg/kg bw per day.

Males fed 1000 mg/kg bw per day and necropsied at 12 months had a slightly increased incidence of hepatocellular adenoma. At 24 months, a statistically significant increase in the number of males with hepatocellular adenoma was seen at 500 ($n = 40$) and 1000 ($n = 41$) mg/kg bw per day, the incidence in controls being 27/50. Low incidences of a variant form of hepatocellular carcinoma (hepatoblastoma) were observed in all treated groups of males (2/50 at 250 mg/kg bw per day, 6/50 at 500 mg/kg bw per day, and 3/50 at 1000 mg/kg bw per day *versus* 0/50 in controls), but the incidence of hepatocellular carcinoma was not significantly increased at any dose. The combined incidence of hepatoblastoma and hepatocellular carcinoma was also not significantly increased in the male mice. The primary non-tumourous microscopic changes in the livers of male mice, which appeared to have been adaptive, ultimately resulted in the promotion of hepatocellular adenomas. The incidences of tumours in other tissues were not statistically significantly increased. The livers of female mice showed similar microscopic adaptive changes, but none of them had hepatoblastoma, and no statistically significant increase in the incidence of tumours in any tissues was found. Decreased incidences of microscopic lesions when compared with controls were found in the adrenals, kidneys, lungs, oral tissues, pancreas, peripheral nerve, spleen, and testis of males and in the kidneys, lungs, and nasal tissues of females. These findings were considered to reflect normal variation and the decreased body weights of the mice and not a primary response to 2-phenylphenol. A NOAEL for toxicity could not be identified. The NOAEL for carcinogenicity was 250 mg/kg bw per day on the basis of an increased incidence of hepatocellular adenomas at 500 mg/kg bw per day (Quast & McGuirk, 1995).

Rats

2-Phenylphenol (purity, 98%) was administered in the diet at concentrations of 0, 6300, 13 000, or 25 000 ppm, equal to 0, 320, 650, and 1300 mg/kg bw per day to groups of 20–24 male Fischer 344 rats (Charles River) for 91 weeks. The percentage survival was 96, 90, 71, and 65% in the four groups, respectively. In the rats that died during the study, the incidences of urinary bladder tumours were 0/1 in controls, 0/2 at 6300 ppm, 7/7 at 13 000 ppm, and 0/8 at 25 000 ppm. Bladder lesions were found in 10%, 96%, and 48% of rats at the three doses, respectively. The incidences of urinary bladder papillomas and transitional-cell carcinomas were 23/24 at 13 000 ppm and 4/23 at 25 000 ppm. A NOAEL could not be identified since bladder lesions were seen at all doses tested (Hiraga, 1983a; Hiraga & Fujii, 1984).

Groups of 70–75 male and 70–75 female Fischer 344 rats were fed diets containing 2-phenylphenol (purity, 99.5%) at concentrations of 0, 800, 4000, or 8000/10 000 ppm, equal to 0, 39, 200, and 400 mg/kg bw per day for males and 0, 49, 240, and 650 mg/kg bw per day for females, for 1 year before interim sacrifice of satellite groups of 20 rats per dose and for 2 years for the remaining 50 rats of each sex. The animals were observed daily and were examined weekly for clinical signs of toxicity. Each animal was weighed once a week and also immediately before necropsy to allow calculation of organ:body weight ratios. Food consumption was measured

weekly. Blood and overnight urine samples were collected at 3, 6, 12, 18, and 24 months from the first 20 surviving rats of each sex in the group scheduled for sacrifice at 2 years. Any dead or moribund animals were prepared for necropsy, and all surviving animals were killed at the end of the test periods.

A 5% decrease in body-weight gain was seen in animals at 4000 ppm, and a decrease of 11% was seen in males at 8000 ppm and in females at 10 000 ppm. Food consumption was unaffected in all groups. Minor clinical and gross observations included an increased incidence of abnormally coloured urine, urine stains, and red stains in male rats given 8000 ppm 2-phenylphenol and an increased incidence of urine and brown stains in female rats given 4000 or 10 000 ppm. There were no treatment-related changes in ophthalmological, haematological, clinical chemical, or urinary parameters, except for an increased incidence of blood in the urine of males at 8000 ppm. The mortality rate was slightly increased among males at 8000 ppm. Gross pathological examination showed increased incidences of urinary bladder masses in males fed 4000 ppm for 2 years or 8000 ppm for 1 or 2 years and increased incidence of pitted zones and abnormal texture of the kidney in females fed 10 000 ppm for 2 years. Histopathological examination showed hyperplasia and transitional-cell carcinoma in the urinary bladders of males fed 4000 or 8000 ppm for 1 or 2 years, the increase being statistically significant at 8000 ppm and of borderline significance at 4000 ppm. The NOAEL for toxicity was 800 ppm, equal to 39 mg/kg bw per day, on the basis of reduced body-weight gain and hyperplasia in the urinary bladder at all doses. The NOAEL for carcinogenicity was 800 ppm, equal to 39 mg/kg bw per day (Wahle & Christenson, 1996).

Sodium 2-phenylphenol

Mice

Sodium 2-phenylphenol (purity, 97%) was administered in the diet to groups of 50 male and 50 female B6C3F$_1$ mice (Charles River) at concentrations of 0, 5000, 10 000, or 20 000 ppm, equal to 0, 590, 1400, and 3000 mg/kg bw per day for males and 0, 780, 1500, and 3100 mg/kg bw per day for females, for 96 weeks. The mice were then given control diet for an additional 8 weeks. The survival rate of males at the high dose was slightly decreased. Decreased body weight was observed in males and females at 20 000 ppm and in females at 5000 and 10 000 ppm. Alkaline phosphatase activity was increased in females at 5000, 10 000, and 20 000 ppm. No urinary bladder stones, tumours, or extensive renal damage were observed in any of the mice. The NOAEL for carcinogenicity was 20 000 ppm, equal to 3000 mg/kg bw per day, the highest dose tested (Ito, 1983a; Hagiwara et al., 1984).

Rats

Groups of 20–21 male and 20–21 female Fischer 344 rats were fed diets containing 0, 1250, 2500, 5000, 10 000, 20 000, or 40 000 ppm of sodium 2-phenylphenol as the tetrahydrate (purity, > 95%), equivalent to 0, 70, 140, 270, 550, 1100, or 2200 mg/kg bw per day, for 91 weeks. The rats were observed daily for changes in general condition. The survival rates were 90, 90, 95, 90, 90, 57, and 71% for the seven groups, respectively. Increased incidences of urinary bladder papillomas and transitional-cell carcinomas were seen, with 1/21 at 5000 ppm, 7/21 at 10 000 ppm, 20/21 at 20 000 ppm, and 17/20 at 40 000 ppm. Transitional-cell carcinomas of the kidney were also observed at doses ≥ 5000 ppm. The NOAEL was 2500 ppm, equivalent to 270 mg/kg bw per day, on the basis of the increased incidence of urinary bladder tumours (Hiraga & Fujii, 1981).

Groups of 50 male and 50 female Fischer 344/DuCrj rats were fed diets containing 0, 7000, or 20 000 ppm (males) or 0, 5000, or 10 000 (females) of sodium 2-phenylphenol (purity, 95.5%) for 104 weeks followed by control diet for 2 weeks. In a second study, groups of 25 male and 25 female rats were fed diets containing the compound at 0, 2500, 7000, or 20 000 ppm, equal to 0, 95, 270, and 770 mg/kg bw per day, for males, and 0, 2500, 5000, or 10 000 ppm, equal to 0, 110, 220, and 470 mg/kg bw per day, for females, for 104 weeks followed by control diet for life.

The survival rate at week 104 was 20% in males at 20 000 ppm in the first study and 24% in the second study, while those in the other groups were > 50%. Urinary bladder tumours were observed in the first study in 2/50 males at 7000 ppm, 47/50 males at 20 000 ppm, 1/50 females at 5000 ppm, and 4/50 females at 10 000 ppm. In the second study, the bladder tumour incidence

was 3/25 in males at 7000 ppm, 23/25 in males at 20 000 ppm, and 2/25 in females at 10 000 ppm. In the first study, transitional-cell carcinomas were found in 2/2 males at 7000 ppm, 46/47 males at 20 000 ppm, and 1/4 females at 10 000 ppm. In the second study, carcinomas were found in 1/3 males at 7000 ppm, 21/23 males at 20 000 ppm, and 1/2 females at 10 000 ppm. The incidence of bladder tumours was thus dose-dependent. The NOAEL was 2500 ppm, equal to 95 mg/kg bw per day, on the basis of urinary bladder tumours at all doses (Hiraga, 1983b; Fujii & Hiraga, 1985).

A working group convened by the International Agency for Research on Cancer (IARC) classified sodium 2-phenylphenol as possibly carcinogenic to humans and 2-phenylphenol as not classifiable as to its carcinogenicity to humans (IARC, 1987, 1999).

(d) Genotoxicity

The results of tests for the genotoxicity of 2-phenylphenol, sodium 2-phenylphenol, and the metabolites phenylhydroquinone and phenylbenzoquinone are summarized in Table 2.

Covalent binding to urinary bladder DNA was determined *in vivo* in pooled samples from eight male rats dosed with 500 mg/kg bw of [^{14}C]2-phenylphenol (purity, 99.8%) or [^{14}C]sodium 2-phenylphenol (purity, 98.7%). No radiolabel was detected in DNA from bladders excised 16 h after dosing with either compound. The detection limit was less than one alkylation per 10^6 nucleotides. Identical results were obtained in a second experiment (Reitz et al., 1983).

The reactions of 2-phenylphenol and its metabolites phenylhydroquinone and phenylbenzoquinone with DNA were investigated by a sequencing technique and by ultraviolet–visible and electron spin resonance spectroscopy. In the presence of Cu(II), phenylhydroquinone caused extensive DNA damage. Catalase, methionine, and methional inhibited the DNA damage completely, whereas mannitol, sodium formate, ethanol, *tert*-butyl alcohol, and superoxide dismutase did not. Phenylhydroquinone plus Cu(II) frequently induced a piperidine-labile site at thymine and guanine residues. Addition of Fe(III), Mn(II), Co(II), Ni(II), Zn(II), Cd(II), or Pb(II) to phenylhydroquinone did not induce DNA damage. This metabolite also induced DNA damage in the presence of Cu(II) when peroxide was added, and Cu(II) accelerated the autoxidation of phenylhydroquinone to quinone. Electron spin resonance spectroscopy revealed that the semiquinone radical is an intermediate in the autoxidation. Catalase did not inhibit the acceleration by Cu(II). Superoxide dismutase promoted both the autoxidation of phenylhydroquinone and the initial rate of semiquinone radical production. Electron spin resonance trapping showed that addition of Fe(III) produced hydroxyl radicals during the autoxidation of phenylhydroquinone, whereas addition of Cu(II) did so sparingly. The results suggest that DNA damage induced by phenylhydroquinone plus Cu(II) is due to active species other than hydroxyl free radicals (Inoue et al., 1990).

DNA adduct formation in HL-60 cells treated with the 2-phenylphenol metabolites 2-phenylhydroquinone and 2-phenylbenzoquinone was studied by ^{32}P-postlabelling. Treatment with 25–500 µmol/L of 2-phenylhydroquinone for 8 h produced one principal and three minor adducts, with a relative distribution of 80, 10, 6, and 4%. The relative adduct frequencies were 0.26–2.3 adducts/10^7 nucleotides. Treatment with 25–250 µmol/L of 2-phenylbenzoquinone for 2 h resulted in a similar level of DNA modification and adduct distribution. Reaction of purified calf thymus DNA with 2-phenylbenzo-quinone produced one DNA adduct, which did not correspond to the major adduct produced in HL-60 cells. These results show that both metabolites can form DNA adducts. Peroxidase activation of 2-phenylphenol may therefore play a role in its carcinogenic effect (Horvath *et al.*, 1992).

In a similar study of covalent binding to DNA, ^{32}P-postlabelling analysis of the products of reaction of DNA with phenylbenzoquinone revealed four major and several minor adducts. Chemical reaction with deoxyguanosine 3′-phosphate also resulted in four major adducts, and their chromatographic mobility was identical to that of major adducts of phenylbenzoquinone–DNA, which were shown to be stable. More total covalent binding was found in deoxyguanosine

Table 2. Results of studies of the genotoxicity of 2-phenylphenol, sodium 2-phenylphenol, and the metabolites phenylhydroquinone and phenylbenzoquinone

End-point	Test object	Concentration	Purity (%)	Result	Reference
2-Phenylphenol					
In vitro					
DNA strand breaks	*E. coli* plasmid	10^{-6}–10^{-2} mol/L	> 99	Negative – S9	Nagai et al. (1990)
DNA ^{32}P-postlabelling	Rat liver DNA	100 µmol/L	NR	Positive + S9 Negative – S9	Pathak & Roy (1992)
DNA binding	Calf thymus DNA	40 mmol/L	> 99	Positive + S9 Negative – S9	Ushiyama et al. (1992)
Gene mutation	*B. subtilis* H17, M45	NR	NR	Negative	Shirasu et al. (1978)
Gene mutation	*S. typhimurium* TA92, TA1535, TA100, TA1537, TA94, TA98	10 -1000 µg/plate	NR	Negative + S9 Negative – S9	Ishidate et al. (1983)
Gene mutation	*E. coli* WP2 *hcr*	NR	NR	Negative + S9 Negative – S9	Shirasu et al. (1978)
Gene mutation	*S. typhimurium* TA100, TA1535, TA1537, TA98	3–200 µg/plate	> 99	Negative + S9 Weakly posi tive + S9	National Toxicology Program (1986)
Gene mutation	Mouse lymphoma (L5178Y) cells, *Tk* locus	0.3–60 µg/ml	> 99	Positive + S9 Positive – S9	National Toxicology Program (1986)
Gene mutation	Human Rsa cells, repair deficient, *HPRT* locus	1–30 µg/ml	NR	Positive	Suzuki et al. (1985)
Chromosomal aberration	CHO-K1 cells	50–175 µg/ml	> 99	Positive – S9	Tayama-Nawai et al. (1984)
Chromosomal aberration	CHO fibroblasts	12–125 µg/ml	NR	Weakly positive + S9 Weakly positive – S9	Ishidate et al. (1983)
Chromosomal aberration	CHO-K1 cells	60–90 µg/ml	> 99	Negative + S9 Negative – S9	National Toxicology Program (1986)
Chromosomal aberration	CHO-K1 cells	25–175 µg/ml	> 99	Positive + S9 Negative – S9	Tayama et al. (1989)
Chromosomal aberration	CHO-K1 cells	100–200 µg/ml	> 99	Positive + S9 inhibited by cysteine or glutathione	Tayama & Nakagawa (1991)
Host-mediated gene mutation	*S. typhimurium* G46 in male JCL-ICR mice	200 or 600 mg/kg bw orally for 5 days	NR	Negative	Shirasu et al. (1978)
In vivo					
Sex-linked recessive lethal mutation	*D. melanogaster*	250 ppm in feed for 3 days or injection of 500 ppm	> 99	Negative	National Toxicology Program (1986)
DNA binding	Male rat urinary bladder	500 mg/kg bw orally	> 99	Negative	Reitz et al. (1983)
DNA ^{32}P-postlabelling	Rat urinary bladder	60–940 mg/kg bw per day	> 99	Positive at 570 and 940 mg/kg bw per day	Christensen et al. (1996a)
Chromosomal aberration	Male rat bone marrow	800 mg/kg bw over 5 days or single doses ≤ 4000 mg/kg bw orally	NR	Negative	Shirasu et al. (1978)
Dominant lethal mutation	Male mice	100 or 500 mg/kg bw per day for 5 days	> 99	Negative	Kaneda et al. (1978)
Dominant lethal mutation	Male mice	100 or 500 mg/kg bw per day for 5 days	NR	Negative	Shirasu et al. (1978)
Sodium 2-phenylphenol					
In vitro					
Gene mutation	*S. typhimurium* TA100, TA98	50–5000 µg/plate	NR	Negative + S9 Negative – S9	Ishidate et al. (1983)
Gene mutation	*S. typhimurium* TA100, TA98, TA1535, TA1537, TA1538	0.025–250 µg/plate	99	Negative + S9 Negative – S9	Reitz et al. (1983)

Table 2 (contd)

End-point	Test object	Concentration	Purity (%)	Result	Reference
Unscheduled DNA synthesis	Male rat primary hepatocytes	10^{-7}–10^{-4} mol/L	99	Negative	Reitz et al. (1983)
In vivo					
DNA ^{32}P-postlabelling	Male rat urinary bladder	2% in diet for 13 weeks	> 99	Positive	Ushiyama et al. (1992)
DNA ^{32}P-postlabelling	Mouse skin	10 or 20 mg/animal topically	97	Positive	Pathak & Roy (1993)
Phenylhydroquinone					
In vitro					
DNA strand breaks	*E. coli* plasmid	10^{-4}–10^{-2} mol/L	> 99	Positive	Nagai et al. (1990)
DNA ^{32}P-postlabelling	Rat liver DNA	100 µmol/L	NR	Positive + S9 Negative – S9	Pathak & Roy (1992)
DNA binding	Calf thymus DNA	40 mmol/L	> 99	Positive – S9	Ushiyama et al. (1992)
Gene mutation	V79 CH lung fibroblast cells, *Hprt* locus, ± arachidonic acid	6–125 µmol/L	NR	Negative	Lambert & Eastmond (1994)
Chromosomal aberration	CHO fibroblast cell line	1–25 µg/ml	NR	Negative + S9 Negative – S9	Ishidate et al. (1983)
Chromosomal aberration	CHO-K1 cells	5–150 µg/ml	> 98	Positive + S9 Negative – S9	Tayama et al. (1989)
Chromosomal aberration	CHO-K1 cells	0.3–30 µmol/L	> 98	Positive, inhibited by cysteine or glutathione	Tayama & Nakagawa (1991)
Sister chromatid exchange	CHO-K1 cells	5–150 µg/ml	> 98	Positive + S9 Positive – S9	Tayama et al. (1989)
Sister chromatid exchange	CHO-K1 cells	0.3–30 µmol/L	> 98	Positive, inhibited by cysteine or glutathione	Tayama & Nakagawa (1991)
Micronucleus formation	V79 CH lung fibroblast cells ± arachidonic acid	6–125 µmol/L	NR	Positive + S9 Negative – S9	Lambert & Eastmond (1994)
In vivo					
DNA damage	Male rat urinary bladder	0.0005–0.1% by injection	99%	Negative	Morimoto et al. (1989)
DNA ^{32}P-postlabelling	Mouse skin	100 µmol/L	NR	Positive + S9 Negative – S9	Pathak & Roy (1992)
Phenylbenzoquinone					
In vitro					
DNA strand breaks	*E. coli* plasmid	10^{-4}–10^{-2} mol/L	> 99	Negative	Nagai et al. (1990)
DNA binding	Calf thymus DNA	40 mmol/L	> 99	Positive – S9	Ushiyama et al. (1992)
Gene mutation	*S. typhimurium* TA100, TA2637, TA98	0.05–1000 µg/plate	NR	PBQ negative/ PBQ negative	Ishidate et al. (1983)
Gene mutation	V79 CH lung fibroblast cells, *Hprt* locus, ± arachidonic acid	6–125 µmol/L	NR	Negative	Lambert & Eastmond (1994)
Chromosomal aberration	CHO fibroblast cell line	1–25 µg/ml	NR	Negative + S9 Negative – S9	Ishidate et al. (1983)
Micronucleus formation	V79 CH lung fibroblast cells ± arachidonic acid	6–125 µmol/L	NR	Negative	Lambert & Eastmond (1994)
In vivo					
DNA damage	Male rat urinary bladder	0.0005–0.1% by injection	99%	Negative	Morimoto et al. (1989)

3'-phosphate than in DNA. Reaction of DNA with 2-phenylphenol or phenylhydroquinone in the presence of microsomes and NADPH or cumene hydroperoxide also resulted in four major adducts, and their formation was drastically decreased by known inhibitors of cytochrome P450. The chromatographic mobility of these adducts matched that of the adducts observed in deoxyguanosine 3'-phosphate and DNA reacted with phenylbenzoquinone. Thus, both 2-phenylphenol and phenylhydroquinone can bind covalently to DNA in the presence of a microsomal cytochrome P450 activation system, and phenylbenzoquinone is one of the DNA-binding metabolites of 2-phenylphenol (Pathak & Roy, 1992).

Covalent modification of skin DNA by sodium 2-phenylphenol *in vivo* was studied by the ^{32}P-postlabelling method to elucidate the biochemical mechanism of promotion of chemically induced skin carcinogenesis by this compound. Topical application of sodium 2-phenylphenol or phenylhydroquinone to the skin of CD-1 mice produced four distinct major and several minor adducts in skin DNA. Total covalent binding in skin DNA was 0.31 fmol/μg DNA after treatment with 10 mg of sodium 2-phenylphenol and 0.62 fmol/μg DNA with 20 mg. The adducts were not observed in skin DNA of untreated animals. Pretreatment of the mice with α-naphthylisothiocyanate, an inhibitor of cytochrome P450, or indomethacin, an inhibitor of prostaglandin synthase, resulted in lower numbers of DNA adducts. Incubation of DNA with 2-phenylphenol or phenylhydroquinone *in vitro* in the presence of cytochrome P450 or prostaglandin synthase activation systems resulted in four major adducts. The pattern of chromatographic mobility observed *in vitro* in the presence of these enzymatic systems appeared to be similar to that of adducts *in vivo*. The chemical reaction of DNA or deoxyguanosine monophosphate with phenylbenzoquinone also resulted in four major and several minor adducts. The four major adducts were identical in chromatographic mobility to the four major adducts produced *in vivo* and *in vitro*. The results show that 2-phenylphenol and phenylhydroquinone can bind covalently to DNA and that one of the DNA-binding metabolites of 2-phenylphenol may be phenylbenzoquinone (Pathak & Roy, 1993).

(e) Reproductive toxicity

(i) Multigeneration reproductive toxicity

2-Phenylphenol

Rats

Groups of 35 albino Sprague-Dawley rats, nine weeks old at the start of the study, were fed diets containing technical-grade 2-phenylphenol (purity, 99.4–99.5%) at concentrations of 200–10 000 ppm, equal to 0, 36, 120, and 460 mg/kg bw per day, for two generations. The dose was adjusted during the premating period according to changes in body weight, and adjustments were made during lactation to avoid overdosing the pups, although this was later considered unnecessary. Two F_1 females at the high dose and 12 F_{2a} pups were removed from the study for examination of the heritability of hypotrichosis. Parents (F_0) for the F_1 generation were assigned randomly to dose groups. The genealogy of the F_{1b} pups was checked to prevent the mating of littermates. For production of the F_2 generation, 132 male and 134 female F_{1b} pups were selected randomly, one or more pups of each sex per litter being used as parents (F_1), divided into 35 pairs of rats per dose, except for the controls which consisted of 27 males and 29 females. Estrus cycles were studied by vaginal smears. The animals were examined daily, and routine observations of birth statistics and pup weights were made; the litters were culled to eight pups when necessary. Standard observations and extensive histological examination of the urinary tract were carried out at sacrifice.

Treatment did not affect clinical signs, body-weight gain during gestation or lactation, or any of the reproductive variables examined. Histological examination of the adults and pups revealed no significant changes in the reproductive tracts. F_0 and F_1 adults at 460 mg/kg bw per day showed a treatment-related decrease in body weight which was not clearly related to a decrease in food consumption, and F_{1b}, F_{2a}, and F_{2b} pups showed a statistically significant decrease in body weight on days 14 and/or 21 of lactation, an effect not seen during the first week of lactation, indicating that development had not been disturbed. The relative weight of the kidneys was increased in a dose-dependent manner, in the absence of changes in other organ weights, in males of the F_0 and F_1 generations. Male rats at 120 and 460 mg/kg bw per day had an increased incidence of calculi

in the urinary tract. Transitional-cell hyperplasia was found in the urinary bladder, defined in the report as 'an area (focal or diffuse) of at least three to four cells thick (of cuboidal cells) in an inflated bladder', whereas normal bladders had a cell thickness of one or two flattened cells. Quantification by simple morphometry indicated a compound-related effect in the bladder in F_0 males and females at 120 and 460 mg/kg bw per day and in F_1 males at 457 mg/kg bw per day. Neoplasms of the urinary tract were found in four rats: one bladder and one ureteric tumour in rats at 125 mg/kg bw per day and two bladder tumours in rats at 457 mg/kg bw per day. The NOAEL for reproductive toxicity was 460 mg/kg bw per day and that for carcinogenicity was 36 mg/kg bw per day (Eigenberg, 1990).

In a similar study, groups of 30 CD Sprague-Dawley rats were fed diets containing technical-grade 2-phenylphenol (purity, 99.5–100%) at concentrations of 200–10 000 ppm, equal to 0, 17, 92, and 460 mg/kg bw per day, for two generations. The dose was adjusted during the premating period according to changes in body weight. The F_0 and F_1 adults received the compound in the diet throughout the study, beginning at 7 weeks of age for the F_0 adults and at weaning for the F_1 adults. The animals received treated feed for 10 weeks before breeding, beginning approximately 2 weeks after weaning of the last F_{1b} litter for F_1 parents. F_0 adults were mated to produce the F_{1a} and F_{1b} litters, and F_1 adults (consisting of randomly selected F_{1b} pups) were mated to produce the F_{2a} and F_{2b} litters. Adult animals were evaluated during the study for effects of 2-phenylphenol on body weight, food consumption, clinical signs, estrous cycling, mating, fertility, length of gestation, and litter size. The offspring were evaluated for effects on sex ratio, viability, body-weight gain, and clinical signs. Gross necropsy was performed on all adults and pups, and the reproductive organs, pituitary, kidneys with ureter attached, urinary bladder, and gross lesions of all F_0 and F_1 adults were evaluated histologically.

At 460 mg/kg bw per day, urine staining was observed in F_0 males and females and F_1 males, urinary bladder calculi were found at necropsy in F_1 adult males, and one F_0 male died from renal failure. At this dose, there was an increase in food consumption by females during lactation, decreased pup weight, and a decrease in the terminal body weight of F_0 and F_1 adult males and females. Histopathological examination of the kidneys revealed debris in the renal pelvis, chronic active inflammation, and increased severity of background lesions in F_0 and F_1 males. Further, transitional-cell hyperplasia (simple, nodular, or papilary) of the bladder, calculi, chronic inflammation of the bladder, and dilatation and hyperplasia of the ureter were seen in F_0 and F_1 males. Two F_1 males at this dose had malignant lymphomas in several tissues. One F_1 female at 92 mg/kg bw per day had a nephroblastoma, and one F_0 male at the high dose and one control F_1 female had a pituitary adenoma. All of these lesions were considered to be incidental to treatment.

There were no treatment-related effects on adult reproductive parameters and no effect on litter size, sex ratio, the number of stillborn pups, pup viability, or clinical signs, no gross lesions in the pups, and no treatment-related effects on the organ weights of adults. The mean live birth indexes (with standard error) were 98 (0.91) in the controls, 99 (0.77) at 17 mg/kg bw per day, 98 (1.3) at 92 mg/kg bw per day, and 98 (0.85) at 460 mg/kg bw per day in the F_{1a} generation; 98 (0.85) in the controls, 98 (1.5) at 17 mg/kg bw per day, 99 (0.71) at 92 mg/kg bw per day, and 99 (0.63) at 460 mg/kg bw per day in the F_{1b} generation; 99 (0.84) in the controls, 98 (1.0) at 17 mg/kg bw per day, 97 (1.4) at 92 mg/kg bw per day, and 100 (0.38) at 460 mg/kg bw per day in the F_{2a} generation; and 97 (1.2) in the controls, 99 (0.78) at 17 mg/kg bw per day, 96 (1.9) at 92 mg/kg bw per day, and 99 (0.46) at 460 mg/kg bw per day in the F_{2b} generation. The differences between the groups were not statistically significant. The NOAEL for reproductive toxicity was 460 mg/kg bw per day, the highest dose tested. The NOAEL for systemic and developmental toxicity was 92 mg/kg bw per day, on the basis of decreased body weight and morphological lesions in the kidneys, urinary bladder, and ureter and a decrease in pup body weight (Eigenberg, 1995).

(ii) Developmental toxicity

2-Phenylphenol and sodium 2-phenylphenol

Mice

Groups of 20–21 pregnant JCL-ICR mice were given 0, 1500, 1700, or 2100 mg/kg bw per day of 2-phenylphenol (purity not given) or 100, 200, or 400 mg/kg bw per day of sodium 2-

phenylphenol (purity not given) by gavage on days 7–15 of gestation. The animals were weighed daily, and any change in their general condition was noted. On day 18 of gestation, the dams were killed and their uteri opened, and the numbers of implantation scars, fetuses that died in early and late stages, and live fetuses were counted. The live fetuses were weighed and sexed and observed for external abnormalities. The number of corpora lutea in each ovary was counted, the major organs were weighed, and examinations were made to determine whether any macroscopic abnormalities were present.

With 2-phenylphenol, body-weight gain was reduced at 1700 and 2100 mg/kg bw per day. Four dams at 1500 mg/kg bw per day, seven at 1700 mg/kg bw per day, and 16 at 2100 mg/kg bw per day died. Pregnancy was confirmed in 5/5 surviving dams at 2100 mg/kg bw per day, 14/14 at 1700 mg/kg bw per day, 14/17 at 1500 mg/kg bw per day, and 20/21 controls, in which implantation was confirmed at the time of sacrifice and laparotomy on day 18 of gestation. The only significant changes found at autopsy of these mice were reduced heart weights at 1700 and 2100 mg/kg bw per day and significantly increased liver weights at 1500 and 1700 mg/kg bw per day, a tendency that was also seen at 2100 mg/kg bw per day. Live fetuses were found in all pregnant dams. The body weights of male and female fetuses at all three doses of 2-phenylphenol were significantly reduced, and the decrease was dose-related in males. No unique external or internal deformities or abnormalities were found in the fetuses, and the skeletal abnormalities found were compatible with delayed development. No NOAEL could be identified for maternal or fetotoxicity. The NOAEL for developmental toxicity was 2100 mg/kg bw per day, the highest dose tested.

With sodium 2-phenylphenol, body-weight gain was statistically significantly reduced in a dose-dependent manner in dams at all doses. Four animals at 200 mg/kg bw per day and 16 at 400 mg/kg bw per day died. No abnormalities were seen at autopsy of dams on day 18 of gestation. Significantly reduced liver, heart, and spleen weights were recorded in animals at 400 mg/kg bw per day, while the weight of the lungs was increased in dams at 200 mg/kg bw per day. Dams at 200 mg/kg bw per day had a low average number of implantations and a low average number of live fetuses. No NOAEL could be identified for maternal toxicity. The NOAEL was 100 mg/kg bw per day for fetotoxicity and 400 mg/kg bw per day, the highest dose tested, for developmental toxicity (Ogata *et al.*, 1978).

2-Phenylphenol

Rats

Groups of 18–20 pregnant Wistar rats were given 0, 150, 300, or 600 mg/kg bw per day of 2-phenylphenol (purity, 99.7%) by gavage on days 6–15 of gestation. An additional group of 11 rats was given 1200 mg/kg bw per day, but this dose proved to be lethal. No untoward signs of toxicity were observed in the controls or at 150 mg/kg bw per day. At doses ≥ 300 mg/kg bw per day, dose-related ataxia and decreased mean body-weight gains were observed. All surviving rats were killed on day 20 of gestation, and the uterine contents were examined. Fetuses were grossly examined; the skeletons were examined with Alizarin red S and the viscera by a modified Wilson method. The mean numbers of implantation sites, live fetuses, resorptions, and fetal weights in animals at 150 and 300 mg/kg bw per day were comparable to those of controls, but at 600 mg/kg bw per day the number of fetal resorptions was increased and fetal weight was decreased. Although a few fetal anomalies were observed in all groups, they did not appear to be related to treatment. The NOAEL was 150 mg/kg bw per day for maternal toxicity, 300 mg/kg bw per day for fetotoxicity, and 600 mg/kg bw per day, the highest dose tested, for developmental toxicity (Kaneda et al., 1978).

In a similar study, groups of 25–35 pregnant rats were given 0, 100, 300, or 700 mg/kg bw per day of 2-phenylphenol (purity, 99.7%) by gavage on days 6–15 of gestation. They were killed on day 21, and the fetuses were removed surgically. All fetuses were weighed, sexed, and examined externally and skeletally, and the soft tissues of approximately one-third of the fetuses were examined. One rat at the high dose died as a result of a dosing accident. Pregnant rats given 700 mg/kg bw per day gained significantly less body weight during the first 4 days of treatment (days 6–9 of gestation) than did controls, and their food consumption was significantly decreased on days 9–11 of gestation. At necropsy, the weights of the liver (but not the liver:body weight ratios) were significantly decreased. There was no effect on the number of implantation sites per

dam, mean litter size, incidences of resorptions, or fetal body weight or crown–rump length. The only major malformation—hypoplastic tail and missing sacral and caudal vertebrae—was observed in a single fetus at 300 mg/kg bw per day. An increase in the incidence of delayed ossification of sternebrae and unossified sternebrae was observed at 700 mg/kg bw per day. The incidences of foramina and bony islands in the skull were also slightly increased in this group. No adverse effects on embryonic or fetal development were observed that were considered to be due to 2-phenylphenol. The NOAEL was 300 mg/kg bw per day for maternal toxicity and 700 mg/kg bw per day, the highest dose tested, for fetotoxicity and developmental toxicity (John et al., 1981).

Rabbits

In a range-finding study, groups of two non-pregnant New Zealand white rabbits were given doses of 0, 100, 500, or 1000 mg/kg bw per day of 2-phenylphenol (purity, 99.8%) in corn oil for 13 consecutive days and were submitted to gross necropsy after the last day. The animals were examined for clinical signs, body weights, body-weight gain, kidney and liver weights, and gross appearance. The rabbits at 1000 mg/kg bw per day appeared to have stopped eating and had lost 24% of their body weight by day 7. One rabbit at this dose died on day 8, and the second was killed in moribund condition on day 10 with nonspecific lesions or lesions secondary to anorexia. Rabbits given 500 mg/kg per day showed a slight decrease in body-weight gain. All the other rabbits survived to the end of the study with no other treatment-related effects. The dose of 100 mg/kg bw per day was tolerated over the course of treatment.

In the second study, groups of seven artificially inseminated females were given 0, 250, 500, or 750 mg/kg bw per day of 2-phenylphenol (purity, 99.8%) in corn oil by gavage on days 7–19 of gestation. They were observed for clinical signs, body weight, and body-weight gain. On day 20 of gestation, all surviving animals were killed and examined for gross pathological alterations and changes in liver and kidney weights. The uteri and ovaries were examined for implantations, resorptions, and corpora lutea, and the liver, kidneys, and stomach were examined histologically. Dose-related signs of maternal toxicity were seen at all doses. One rabbit at 250 mg/kg bw per day, two at 500 mg/kg bw per day, and six at 750 mg/kg bw per day died. The dose-related effects observed included increased incidences of haemorrhage, gaseous distension, and erosions of the stomach, decreased or soft ingesta in the gastrointestinal tract, decreased body weight and body-weight gain, increased absolute and relative mean weights of the kidney, and increased incidence and/or severity of renal tubular degeneration and inflammation. Treatment-related effects were observed on reproductive, embryonal, or fetal parameters at 750 mg/kg bw per day.

In the third study, groups of 16–24 artificially inseminated adult female New Zealand white rabbits were given 0, 25, 100, or 250 mg/kg bw per day of 2-phenylphenol (purity, 99.8%) in corn oil by gavage on days 7–19 of gestation. They were observed for clinical signs, body weight, and body-weight gain. On day 28 of gestation, all surviving rabbits were killed and necropsied, when the weights of the liver, kidney, and gravid uterus and the numbers of corpora lutea, implantations, resorptions, and live and dead fetuses were recorded. All fetuses were removed from the uterus, weighed, sexed, and examined for external, visceral, and skeletal alterations. The kidneys of all animals were examined histologically. Administration at 250 mg/kg bw per day resulted in maternal toxicity evidenced by treatment-related mortality (13%), gross pathological alterations (ulceration and haemorrhage of the gastric mucosa, haemolysed blood in the intestinal tract, and decreased ingesta), and histopathological alterations (renal tubular degeneration and inflammation). No significant maternal effects were observed at 25 or 100 mg/kg bw per day, and no adverse embryonal or fetal effects were observed at any dose. The overall NOAELs were 100 mg/kg bw per day for maternal toxicity, 500 mg/kg per day for fetotoxicity, and 750 mg/kg per day, the highest dose tested, for developmental toxicity (Zablotny et al., 1991).

(f) Special studies: Mechanisms of carcinogenicity in rat urinary bladder

In a study to determine whether 2-phenylphenol is a complete skin carcinogen or a promoter in a two-stage initiation and promotion process, the compound was applied to the interscapular area of the backs of 50 male and 50 female Swiss CD-1 mice at a dose of 55.5 mg in 0.1 ml acetone, three times per week for 2 years. A second group of 50 male and 50 female mice was treated identically except that their backs were pretreated once with 0.05 mg in 0.1 ml acetone of 7,12-

dimethylbenz[*a*]anthracene (DMBA), a known initiator of skin cancer. Additional groups of 50 male and 50 female mice served as acetone vehicle controls, controls treated once with DMBA and thereafter only with acetone, and a positive control group treated once with DMBA and thereafter with 12-*O*-tetradecanylphorbol 13-acetate (TPA), a known promoter of skin cancer, at a dose of 0.005 mg in 0.1 ml acetone, three times per week for 2 years.

The mean body weights and survival of the mice treated with 2-phenylphenol or with DMBA plus 2-phenylphenol were generally similar to those of the respective negative control groups, but the survival of the group given DMBA plus TPA was substantially decreased. In this group, the incidences of squamous-cell papillomas and carcinomas, keratocanthomas, and basal-cell carcinomas at the site of application were clearly increased (52/100) over that in the group given DMBA plus acetone(15/100). The time to tumour was also substantially decreased in the group given DMBA plus TPA. Similar neoplastic skin lesions were observed with DMBA plus acetone (17/100), but at an incidence equivalent to that in the control group (15/100). No neoplastic skin lesions were observed in the group given 2-phenylphenol. The author concluded that 2-phenylphenol is not carcinogenic alone or as a promoter (Luster, 1986).

The promoting effect of 2-phenylphenol (purity, 98%) and sodium 2-phenylphenol (purity, 97%) in the urinary bladder was studied in male Fischer 344 rat initiated with *N*-nitrosobutyl-*N*-(4-hydroxybutyl)amine (NBHBA). Groups of 30 rats were given drinking-water containing 0.01% NBHBA for 4 weeks and then diets containing 20 000 ppm of sodium 2-phenylphenol (equivalent to 1000 mg/kg bw per day) for 32 weeks, NBHBA for 4 weeks followed by untreated feed for 32 weeks, or drinking-water without NBHBA for 4 weeks followed by diet containing 20 000 ppm of sodium 2-phenylphenol for 32 weeks. In another experiment, groups of 30 male rats were given 0.05% NBHBA in drinking-water for 4 weeks followed by diets containing 20 000 ppm of sodium 2-phenylphenol or 20 000 ppm of 2-phenylphenol for 32 weeks, no NBHBA for 4 weeks, and then 20 000 ppm of sodium 2-phenylphenol (15 rats) or 20 000 ppm of 2-phenylphenol (15 rats) in the diet for 32 weeks. In a third experiment, groups of 15 rats were given diets containing 0, 20 000 ppm of sodium 2-phenylphenol, or 20 000 ppm of 2-phenylphenol. Urine samples were obtained from these rats by forced urination on days 27, 29, and 32.

Administration of 20 000 ppm sodium 2-phenylphenol in the diet significantly increased the incidence and number of preneoplastic lesions (papillary or nodular hyperplasia) per 10 cm of basement membrane of the urinary bladder in male rats pretreated with 100 ppm NBHBA, and the incidence and number of papillomas and carcinomas of the urinary bladder in the group pretreated with 500 ppm NBHBA. Moreover, treatment with sodium 2-phenylphenol alone, without initiation, induced papillary or nodular hyperplasia, papillomaa, and carcinoma. In contrast, administration of 2-phenylphenol in the diet after initiation only slightly increased the incidence of urinary bladder lesions over that with NBHBA alone, and its effect was not statistically significant. No tumours of the urinary bladder were induced by 2-phenylphenol alone. The authors concluded that sodium 2-phenylphenol, and not 2-phenylphenol, has tumour promoting activity and might be a complete carcinogen in rat urinary bladder. Since the sodium salt increased the pH of the urine, the authors speculated that an active metabolite reaches the urinary bladder at a higher concentration than with 2-phenylphenol. They suggested that sodium 2-phenylphenol is a carcinogens that acts by a non-genotoxic mechanism (Fukushima et al., 1983).

In a similar study, 2-phenylphenol or sodium 2-phenylphenol (purity of neither given) was administered in the diet at a concentration of 20 000 ppm to 28 male Fischer 344 rats for 64 weeks. One rat receiving sodium 2-phenylphenol had small stones in the urinary bladder, and this compound, but not 2-phenylphenol, induced papillary or nodular hyperplasia (19/28), papillomas (5/28), and carcinomas (6/28) of the urinary bladder. Pretreatment of additional rats with NBHBA increased the incidence of papillary or nodular hyperplasia ($p < 0.05$), papillomas (not significant), and carcinomas (not significant) over that in rats treated with NBHBA alone. In another experiment, 2-phenylphenol or sodium 2-phenylphenol was administered in the diet at concentrations of 2500, 5000, 10 000, or 20 000 ppm to groups of five to nine male Fischer 344 rats for up to 104 weeks. Animals from each group were killed and examined at 4, 8, 12, 24, 36, and 104 weeks. No stone formation was observed in the urinary bladders of rats treated with sodium 2-phenylphenol. At 20 000 ppm, simple hyperplasia of the urinary bladder was observed

from 4 weeks in 5/5 animals, papillary or nodular hyperplasia from 36 weeks in 5/5 animals, and papillomas in 2/5 and carcinomas in 2/5 at 104 weeks. At 10 000 ppm of sodium 2-phenylphenol, only simple hyperplasia was observed from 36 weeks. 2-Phenylphenol alone did not cause bladder tumours and did not enhance the bladder lesions induced by NBHBA (Ito, 1983b).

In a short-term assay for bladder carcinogenicity in rats, increased agglutinability of bladder epithelial cells with concanavalin A was observed after a 1-week treatment with 10 000 or 20 000 ppm of 2-phenylphenol or sodium 2-phenylphenol (purity of neither given), suggesting that these compounds cause bladder cancer. No such increase was observed in rats fed diets containing ρ-phenylphenol or biphenyl derivatives at 20 000 ppm. In male Fisher rats fed diets containing 20 000 ppm of sodium 2-phenylphenol for 50 weeks, bladder papillomas developed in 19 of 36 rats and bladder carcinomas in 14 of 36 rats (Honma et al., 1983).

Groups of 20 male Fischer 344 rats were fed diets containing 20 000 ppm of 2-phenylphenol, 20 000 ppm of sodium 2-phenylphenol (purity of neither given), or 5000 ppm of biphenyl for up to 24 weeks. Changes in the amounts of DNA synthesis and in the morphology of the renal papilla and renal pelvis were recorded under light and scanning electron microscopes. Increased DNA synthesis in both renal papilla and pelvis and distinct morphological alterations in the cell surface were seen with 2-phenylphenol and its sodium salt by 4 weeks. Sequential light microscopy revealed renal papillary necrosis in animals fed 2-phenylphenol from week 4, followed by regenerative hyperplasia at weeks 16 (1/5) and 24 (3/5), but no changes in the renal pelvis. Feeding of sodium 2-phenylphenol caused similar changes in the renal papillae and also hyperplasia in the renal pelvis (2/5). No proliferative response of the kidney was apparent in rats fed biphenyl. The authors concluded that the proliferative responses caused by sodium 2-phenylphenol in the renal pelvic epithelium were similar to those induced by this compound in the urinary bladder (Shibata et al., 1989a).

The interactive effects of ascorbic acid, saccharin, and hippuric acid on the carcinogenicity of 2-phenylphenol and sodium 2-phenylphenol were studied in groups of 20 male Fischer 344 rats. The animals were given 2-phenylphenol (purity, 99.5%) or sodium 2-phenylphenol in the diet at a concentration of 20 000 ppm (equivalent to 1000 mg/kg bw per day) for 24 weeks with or without ascorbic acid, sodium ascorbate, acid saccharin, sodium saccharin, hippuric acid, or sodium hippurate at 50 000 ppm. The urinary sodium concentration was increased in all animals receiving sodium salts and/or sodium 2-phenylphenol. The pH of the urine was increased in those given sodium 2-phenylphenol, sodium ascorbate, or sodium saccharin, and the osmolality was decreased in those given sodium 2-phenylphenol, sodium ascorbate, or sodium hippurate. 2-Phenylphenol decreased the osmolality but did not affect the pH or sodium concentration of urine. Histopathologically, the bladders of rats given sodium 2-phenylphenol showed epithelial thickening (epithelial thickness, four to eight cells) at 8, 16, and 24 weeks and papillary and nodular changes at 16 and 24 weeks. Treatment with the other sodium salts provoked 'slight to moderate' hyperplasia at 8 and 16 weeks but no papillary or nodular changes; the changes had regressed by 24 weeks. The combination of raised urinary pH and sodium promoted the effects of sodium 2-phenylphenol, while sodium hippurate raised urinary sodium but not pH and had no effect (Fukushima et al., 1989).

In an essentially similar study, groups of 31 male Fischer 344 rats received $NaHCO_3$ to raise the urinary pH or NH_4Cl to lower it. 2-Phenylphenol was given at a dietary concentration of 12 500 ppm (equivalent to 625 mg/kg bw per day) and sodium 2-phenylphenol at 20 000 ppm (equivalent to 1000 mg/kg bw per day or 625 mg/kg bw per day of 2-phenylphenol). Hyperplasia of the bladder epithelium was seen in animals given 2-phenylphenol, 2-phenylphenol plus $NaHCO_3$, or sodium 2phenylphenol. Administration of the sodium salt with NH_4Cl had no significant effect. The incidence of tumours was significantly increased with 2-phenylphenol (12/31), sodium 2-phenylphenol (22/31), and 2-phenylphenol plus $NaHCO_3$ (20/31), but only three tumours were seen in 31 rats given sodium 2-phenylphenol plus NH_4Cl. Thus, the carcinogenic effects of 2-phenylphenol were promoted in alkaline urine, and those of sodium 2-phenylphenol were inhibited in acid urine (Fujii et al., 1987).

Changes in urinary parameters, particularly electrolyte levels and pH, DNA synthesis, and the morphology of the bladder epithelium were investigated in Fischer 344 rats fed diets containing various sodium, potassium, magnesium, and calcium carbonate salts at a concentration of 30 000 ppm, with or without L-ascorbic acid at 50 000 ppm, for 4 or 8 weeks. The effects of treatment with NH_4Cl at 10 000 ppm (to acidify urine) and of combined treatment with sodium ascorbate at 50 000 ppm and NH_4Cl were also investigated. Urinary pH was significantly raised in groups given $NaHCO_3$, K_2CO_3, ascorbic acid plus $NaHCO_3$, ascorbic acid plus K_2CO_3, or sodium ascorbate, whereas treatment with ascorbic acid or NH_4Cl alone caused a significant decrease in urinary pH. Increases in urinary electrolyte or ascorbic acid contents were associated with the corresponding dosing regimen. DNA synthesis in the bladder epithelium was increased in groups given $NaHCO_3$, K_2CO_3, ascorbic acid plus $NaHCO_3$, ascorbic acid plus K_2CO_3, or sodium ascorbate. Furthermore, all treatments that increased DNA synthesis also induced some morphological alterations in the bladder epithelium. Administration of ascorbic acid in conjunction with $NaHCO_3$ or K_2CO_3 induced more changes than those with either salt alone. In contrast, the degree of response of the bladder epithelium of rats given sodium ascorbate was reduced by simultaneous administration of NH_4Cl. These results suggest that the degree of DNA synthesis and/or morphological alteration in rat bladder epithelium after treatment with various bases depends on changes in the urinary concentrations of Na^+ or K^+ and/or pH and the presence of ascorbic acid in the urine (Shibata et al., 1989b).

The role of urinary pH and Na^+ concentration on the carcinogenic effect of 2-phenylphenol and sodium 2-phenylphenol on rat urinary bladder was studied in two experiments. In the first, groups of 36 male Fischer 344 rats were fed diets containing 20 000 ppm of sodium 2-phenylphenol (equivalent to 1000 mg/kg bw per day), 12 500 ppm of 2-phenylphenol (equivalent to 625 mg/kg bw per day), 6400 ppm of $NaHCO_3$, 12 500 ppm of 2-phenylphenol plus 6400 ppm of $NaHCO_3$, 12 500 ppm of 2-phenylphenol plus 3200 ppm of $NaHCO_3$, or 12 500 ppm of 2-phenylphenol plus 1600 ppm of $NaHCO_3$ for 104 weeks. Body weights were measured weekly up to week 14 and monthly thereafter. Food consumption was measured on 2 consecutive days per week on a per-cage basis. Urine samples were obtained from four to six rats in each group by forced urination, and the urinary pH was determined 10 times during the 2-year experiment. For measurement of urinary electrolytes, three or four rats in each group were housed individually in metal metabolic cages without food or water for 4 h in the morning during weeks 58, 80, and 96. All surviving animals were killed at the end of the experiment and were examined carefully for gross abnormalities at autopsy. The liver, kidney, and tissues with macroscopic lesions were removed and fixed for histological examination. Autopsies were also performed on all animals that died or became moribund and were killed during the experiment.

Body-weight gain was reduced throughout the study in all treated groups, but the reduction was less and started later in animals given 6400 ppm of $NaHCO_3$ alone. The absolute and relative weights of the bladder were significantly higher in treated groups than in controls, especially in rats given 2-phenylphenol plus 6400 ppm of $NaHCO_3$. The relative weights of the kidneys and liver in all treated groups were significantly higher than in controls. At week 104, 58% of rats given sodium 2-phenylphenol and 68–84% of those in other groups were still alive compared with 73% of controls. Macroscopically, more tumours were found in bladders of rats fed sodium 2-phenylphenol or 2-phenylphenol plus 6400 ppm of $NaHCO_3$ than in rats fed 2-phenylphenol plus 3200 ppm or 1600 ppm $NaHCO_3$, and no tumours were found in rats fed 2-phenylphenol alone or in controls. No stone formation related to tumours was seen in any group. The bladder lesions were classified as simple hyperplasia, papillary or nodular hyperplasia, papilloma, and carcinoma. The incidences of bladder carcinomas were significantly higher than controls in rats fed sodium 2-phenylphenol or 2-phenylphenol plus 6400 ppm $NaHCO_3$.

In the other experiment, groups of five rats were given diets supplemented with test chemicals as in the first experiment for only 8 weeks before being killed. Urinary electrolytes and pH were determined at weeks 2, 4, 6, and 8, and osmolality was measured at weeks 4 and 8. When all animals were killed at week 8, no stone formation was observed macroscopically in any groups. Various changes in the luminal surface of the bladder, particularly in rats fed sodium 2-phenylphenol or 2-phenylphenol plus 6400 ppm of $NaHCO_3$, were revealed by scanning electron microscopy. The authors concluded that sodium 2-phenylphenol is carcinogenic to the male rat

bladder at 20 000 ppm in the diet. 2-Phenylphenol was not carcinogenic, although it induced a low incidence of papillary or nodular hyperplasia (Fukushima et al., 1989).

Species differences in the induction of urinary bladder lesions by sodium 2-phenylphenol were studied in groups of 30 male Fischer 344 rats, B6C3F$_1$ mice, Syrian golden hamsters, and Hartley guinea-pigs fed diets containing 20 000 ppm of sodium 2-phenylphenol (purity not given). Body weight and food consumption were determined periodically. Groups of five animals from each group were killed at weeks 4, 8, 12, 24, 36, and 48, and five control animals were killed at weeks 12 and 48. Urine was collected in metabolism cages for 4 h from all animals at weeks 12 and 48 for measurement of urine volume, pH, osmolality, and microscopic appearance. Although food consumption did not differ between the treated and untreated groups, retardation of growth was associated with administration of sodium 2-phenylphenol in the diet, especially during the first 8 weeks of the test. Although the absolute weights of the liver were similar in both treated and untreated groups, the relative weights were slightly increased in all species. Morphological changes in the urinary bladder were remarkable only in rats, which showed simple hyperplasia at week 4, increasing in incidence and density to week 48. Lesions classified as papillary nodular hyperplasia were observed in rats from week 36 of treatment, but no papillomas were found. Scanning electron microscopy revealed pleomorphic microvilli only in rats, which increased in grade with time. In the other species, no changes indicative of proliferation were observed, except for a slight effect in mice at weeks 24 and 48. The pH of the urine was slightly increased in rats, while the background pH in the other species was usually high, except in mice at 48 weeks. Osmolality was not affected by administration of sodium 2-phenylphenol, but crystal formation was seen in rats and guinea-pigs, which increased slightly in rats with time. The authors concluded that sodium 2-phenylphenol is likely to be a urinary bladder carcinogen in rats but not in mice, guinea-pigs, or hamsters (Hasegawa et al., 1990a).

Sex differences in the carcinogenic effect in rat urinary bladder associated with administration of 2-phenylphenol (purity, > 99%) and sodium 2-phenylphenol (purity, > 99%) were investigated in groups of five or six male and five or six female Fischer 344 rats fed diets containing 12 500 ppm of 2-phenylphenol (equivalent to 625 mg/kg bw per day), 20 000 ppm of sodium 2-phenylphenol, equivalent to 1000 mg/kg bw per day), 30 000 ppm of $NaHCO_3$, 10 000 ppm of NH_4Cl, 2-phenylphenol plus $NaHCO_3$, or sodium 2-phenylphenol plus NH_4Cl for 8 weeks. Body weights and food and water consumption were determined weekly. Fresh urine specimens were obtained from all rats by forced urination at week 8 and examined for pH and osmolality. During the last week of the study, the rats were transferred to metal metabolism cages without food or water, and urine samples were collected from 07:00–13:00 h over 3 consecutive days to obtain enough pooled urine for analysis and determination of metabolites, although only pooled samples from the groups treated with 2-phenylphenol or its sodium salt were examined.

No animals died before the end of the experiment, and food intake was not significantly different between groups. The body weights at week 8 were significantly lower in all treated groups of male rats and in female rats given 2-phenylphenol or sodium 2-phenylphenol. The urinary pH values for all groups were significantly different from the 7.0 found in untreated males and the 6.8 found in untreated females, except in the groups given sodium 2-phenylphenol alone in which the pH values were comparable to those of controls. The pH values were highest in the groups given $NaHCO_3$ alone, followed by the groups given 2-phenylphenol plus $NaHCO_3$, while the pH was lower than in controls for groups given 2-phenylphenol alone, sodium 2-phenylphenol plus NH_4Cl, or NH_4Cl alone. The Na^+ concentrations were higher in males than in females in all groups except controls and those given NH_4Cl. In the groups given sodium 2-phenylphenol alone, the Na^+ concentration was slightly increased over control values in males but not in females. The depressive effect of NH_4Cl on Na^+ concentration was also less pronounced in females. Only unconjugated urinary metabolites were identified. No urothelial hyperplastic changes were observed with 2-phenylphenol alone in either sex, while an equimolar dose of sodium 2-phenylphenol induced mild papillary and nodular hyperplasia or simple hyperplasia in male rats only. The possible mechanisms underlying the differences in response between the sexes might include excretion of other types of conjugated forms and the formation of microcrystals such as the silicate crystals found in the urine of rats fed sodium saccharin (Hasegawa et al., 1991).

The physiological effects of 2-phenylphenol (purity, 99.5%) on urothelial cells and potential formation of DNA adducts were studied in male Fischer 344 rats. In an initial experiment, rats were fed dietary concentrations of 0, 1000, 4000, or 12 500 ppm for 13 weeks. There was no evidence of urinary calculi, microcrystalluria, or calcium phosphate-containing precipitate, but urothelial cytotoxicity and hyperplasia were seen at the highest dose. In a second experiment, rats were fed dietary concentrations of 0, 800, 4000, 8000, or 12 500 ppm for 13 weeks. The urinary pH was > 7 in all groups. The urinary volume was increased at the highest dose, with consequent decreases in osmolality and the concentrations of creatinine and other solutes. The urinary excretion of total 2-phenylphenol metabolites was increased. Most of the metabolites were conjugates of 2-phenylphenol and of phenylhydroquinone, and free 2-phenylphenol and metabolites accounted for < 2% at each dose. Urothelial toxicity and hyperplasia occurred only at 8000 and 12 500 ppm. No 2-phenylphenol–DNA adducts were detected in the urothelium at any dose. The small percentage of unconjugated metabolites and the absence of DNA adducts suggest that 2-phenylphenol acts as a bladder carcinogen in male rats by inducing cytotoxicity and hyperplasia without direct binding of the compound or its metabolites to DNA (Smith et al., 1998).

The carcinogenic effect of sodium 2-phenylphenol and its metabolites on female rat urinary bladder after intravesicular instillation was studied in groups of nine 6-week-old Fischer 344 rats that received 0.2 ml of a saline solution of 0.1% sodium 2-phenylphenol (purity, > 99%), phenylbenzoquinone (purity, > 99%), or phenylhydroquinone (purity, > 99%) through a catheter into the urethra once, twice, or four times. The pH values of the solutions were 11 for sodium 2-phenylphenol, 6.5 for phenylbenzoquinone, and 6.4 for phenylhydroquinone. Saline or a solution of NaOH (pH 11) were given to controls. The animals were maintained under light ether anaesthesia during instillation and for a further 10 min thereafter to prevent spontaneous urination. Two or three animals from each group were killed under ether anaesthesia at 24 h and 4 and 7 days after the last instillation. The histopathological findings in rats killed 24 h after a single injection of saline, phenylbenzoquinone, or phenylhydroquinone included swelling and vacuolation of urothelial cells. The bladder epithelium of rats treated with alkaline solutions of sodium 2-phenylphenol or NaOH showed minimal hyperplasia associated with mild oedema and inflammatory-cell infiltration of the epithelial and submucosal tissues. Moderate epithelial hyperplasia was seen in rats killed 7 days after treatment with phenylbenzoquinone. In rats given two or four instillations of this metabolite, the grading of the hyperplastic changes was clearly dependent on the number of instillations and the time between the last treatment and death. The epithelial hyperplasia was marked and was classified as papillary and/or nodular in rats treated with four instillations of phenylbenzoquinone and killed 4 days after the last instillation. No carcinomas were induced.

In another experiment, groups of 20 female rats were treated in the same way but twice a week for 5 weeks. From week 6, some rats were fed the basal diet supplemented with 5% sodium saccharin for 31 weeks as a promotion treatment, while the other rats were maintained on basal diet during this period. A separate group was given 500 ppm of NBHBA for 4 weeks, and then 50 000 ppm of sodium saccharin as a positive control. Body weights and food consumption were determined periodically. The histopathological findings in the positive control group included papillomas in two rats, papillary and/or nodular hyperplasia in nine rats, and simple hyperplasia in 11 rats. In contrast, no hyperplastic changes were seen in rats treated first with sodium 2-phenylphenol or its metabolites followed by promotion with sodium saccharin, except in nine rats given phenylbenzoquinone, which had papillary and/or nodular hyperplasia and/or simple hyperplasia. Formation of lymph follicles in the submucosa of the urinary bladder was seen in particular with phenylbenzoquinone and in the positive control group. The authors concluded that phenylbenzoquinone plays an essential role in the urinary bladder carcinogenesis induced by sodium 2-phenylphenol (Hasegawa et al., 1990b).

A series of studies was carried out on the carcinogenicity of 2-phenylphenol and sodium 2-phenyl-phenol (purity of neither given) in rat urinary bladder. In the first study, groups of 10 male Fischer 344 rats were fed diets containing sodium 2-phenylphenol at concentrations of 0, 2500, 5000, 10 000, or 20 000 ppm for 36 weeks. The rats were observed daily and were weighed periodically. Body-weight gain was suppressed at 20 000 ppm. No calculi or mucosal tumours were found grossly, but histological analysis revealed a statistically significant increase in the

frequency of bladder lesions in rats at 20 000 ppm, in which simple hyperplasia was seen in 10/10 and papillary or nodular hyperplasia in 4/10 animals. Simple hyperplasia was seen in 1/10 rats at 10 000 ppm.

In the second study, groups of five male rats were fed diets containing 20 000 ppm of 2-phenylphenol or sodium 2-phenylphenol for 4 weeks. The animals were weighed at the end of treatment, at which time the mean body weights of both treated groups were reduced, to 86% of the control value with 2-phenylphenol and to 96% with sodium 2-phenylphenol. One hour before sacrifice, each rat was given an intraperitoneal injection of 100 mg/kg bw of 5-bromo-2'-deoxyuridine (BrdU), and the urinary bladders were stained immunohistochemically with anti-BrdU antibodies to investigate the capacity of the epithelial cells for proliferation. The number of cells that had taken up BrdU per 1000 urinary bladder epithelial cells was determined by light microscopy and expressed as per cent labelled cells. Rats fed sodium 2-phenylphenol showed increased urinary pH and extensive BrdU-labelling in urinary bladder epithelial cells, indicating increased DNA synthesis. Rats fed 2-phenylphenol also showed a tendency to increased BrdU-labelling, suggested that it also can cause, albeit weak, urinary bladder epithelium proliferation.

In the third study, groups of five male Fischer 344 rats were given diets containing 6400 ppm of $NaHCO_3$; 13 000 ppm of 2-phenylphenol; 13 000 ppm of 2-phenylphenol plus $NaHCO_3$ at 1600, 3200, or 6400 ppm; or 20 000 ppm of sodium 2-phenylphenol for 8 weeks. The urinary pH at week 8 was significantly increased in animals fed $NaHCO_3$ alone, 2-phenylphenol plus 3200 or 6400 ppm $NaHCO_3$, or sodium 2-phenylphenol. The urinary concentrations of Na^+ were significantly increased at 2, 4, 6, and 8 weeks in rats fed sodium 2-phenylphenol and at 8 weeks in rats fed $NaHCO_3$ alone or 2-phenylphenol plus 3200 or 6400 ppm $NaHCO_3$. At 8 weeks, a significant increase or a tendency to an increase in urine volume and significantly lower osmotic pressure were seen in pooled urine samples from all treated groups when compared with controls. When the bladders were examined by scanning electron microscopy, the surface of the epithelium appeared normal in the controls and in animals given only $NaHCO_3$, and was made up of polygonal cells of uniform dimensions with reticular peaked microridges at the surface. In treated animals, the cells in the outermost layer of the bladder epithelium assumed a cobblestone configuration in pavement form; at high magnification, pleomorphic microvilli, short uniform microvilli, and ropy or leafy microridges were seen at the surface of these cells. These alterations were observed mainly in rats given sodium 2-phenylphenol or 2-phenylphenol plus 6400 ppm $NaHCO_3$. The extent of the changes and the frequency of their appearance was correlated with the concentration of $NaHCO_3$ given with 2-phenylphenol.

In the fourth study, groups of 30–31 male Fischer 344 rats were given 20 000 ppm of sodium 2-phenylphenol or 13 000 ppm of 2-phenylphenol with or without $NaHCO_3$ at 1600, 3200, or 6400 ppm for 104 weeks. The animals were observed daily for deaths, and body weight and feed consumption were measured at regular intervals. Pooled 4-h urine samples were collected from three or four rats at each dose at weeks 58, 80, and 96 for measurement of electrolytes. The urinary pH was significantly increased in rats fed sodium 2-phenylphenol or 2-phenylphenol alone or in combination with 3200 or 6400 ppm $NaHCO_3$ or 6400 ppm $NaHCO_3$ alone. The Na^+ concentrations were significantly increased in rats fed sodium 2-phenylphenol, 2-phenylphenol plus 6400 ppm $NaHCO_3$, or 6400 ppm $NaHCO_3$ alone. No difference was seen in rats fed sodium 2-phenylphenol or 2-phenylphenol plus 6400 ppm $NaHCO_3$. Urinary bladder tumours were found in all groups except those given 2-phenylphenol alone, and the frequencies were highest with sodium 2-phenylphenol and with 2-phenylphenol plus 6400 ppm $NaHCO_3$. The presence of calculi could not be confirmed. Histological examination of the urinary bladder epithelium revealed simple hyperplasia, papillary or nodular hyperplasia, papillomas, and carcinomas. Carcinomas occurred in rats fed sodium 2-phenylphenol (41%), 2-phenylphenol plus 6400 ppm $NaHCO_3$ (31%), and 6400 ppm $NaHCO_3$ alone. The results confirm that administration of 20 000 ppm of sodium 2-phenylphenol is carcinogenic in male rats, while an equimolar concentration of 2-phenylphenol causes only a low frequency of papillary or nodular hyperplasia and no papillomas or cancers. Administration of $NaHCO_3$ in conjunction with 2-phenylphenol caused carcinomas, correlated to the $NaHCO_3$ concentration, which also increased urinary pH and Na^+ concentration (Inoue, 1993).

The induction of DNA damage in the urinary bladder epithelium of male and female Fischer 344 rats by 2-phenylphenol and its metabolites was studied by the alkaline elution assay after an

intravesicalar injection. Phenylbenzoquinone at 0.05–0.1% had weak DNA-damaging activity in animals of each sex, whereas 2-phenylphenol and phenylhydroquinone had no effect at the same dose. Histopathological examination revealed diffuse, moderate, simple hyperplasia 5 days after injection of 0.1% phenylbenzoquinone in male rats. The lesions were associated with submucosal infiltration, small round cells, and slight oedema. The only change in the bladders of rats injected with 0.1% phenylhydroquinone was slight swelling and/or vacuolization of the epithelial cells, and the bladders of rats injected with 0.1% 2-phenylphenol were normal (Morimoto et al., 1989).

Groups of 5–10 Fischer 344 rats received diets containing sodium 2-phenylphenol at concentrations of 0, 2500, 5000, 10 000, or 20 000 ppm (equivalent to 0, 250, 500, 1000, and 2000 mg/kg bw per day) for up to 5 months to investigate the correlation between urinary phenylbenzoquinone and DNA damage in the bladder epithelium. Slight but dose-dependent DNA damage was observed in the epithelium of male rats fed 10 000 or 20 000 ppm for 3–5 months. A plot of the dose–response relationship for DNA damage at 3 months showed a threshold at 5000 ppm of sodium 2-phenylphenol. The amounts of unconjugated 2-phenylphenol, phenylhydroquinone, and phenylbenzoquinone in 24-h urine samples collected from males and females after 5 months correlated well with the dietary concentrations of sodium 2-phenylphenol. The total amounts of free metabolites in the urine of males given 5000 ppm were similar to those in the urine of females given 20 000 ppm. Free metabolites represented 0.3% of the total average intake of male rats fed 5000 ppm, 0.8% of the intake of 10 000 ppm, and 1% of the intake of 20 000 ppm of sodium 2-phenylphenol. The average concentrations of free phenylhydroquinone in the urine of males given 20 000 ppm of sodium 2-phenylphenol were significantly higher than those in males fed 5000 ppm or in females fed 20 000 ppm. The concentrations of phenylbenzoquinone were much lower than those of phenylhydroquinone. Only 10% of phenylbenzoquinone was recovered from spiked urine, indicating that this metabolite may react with urinary nucleophilic groups. The authors concluded that phenylbenzoquinone is the reactive species in the initiation of bladder tumours induced by 2-phenylphenol and sodium 2-phenylphenol (Morimoto et al., 1989).

The interaction of 2-phenylphenol and its metabolites with pUC18 DNA from *Escherichia coli* plasmids was studied *in vitro*. The major metabolite formed from 2-phenylphenol by mixed-function oxidases was phenylhydroquinone. This finding corroborates earlier reports that phenylhydroquinone in the form of a glucuronide conjugate is the main product in the bladders of rats fed 2-phenylphenol. When pUC18 DNA was incubated with phenylhydroquinone, DNA strand scission was observed, whereas barely detectable DNA cleavage was seen with 2-phenylphenol and phenylbenzoquinone. DNA cleavage by phenylhydroquinone was inhibited by superoxide dismutase, catalase, and several oxygen radical scavengers, indicating that the oxygen radicals generated in the process of oxidation of phenylhydroquinone in aqueous solution are responsible for the DNA cleavage. The attack seemed to occur at guanine residues in general and was not restricted to guanines with specific residues, indicating no hot spots (Nagai et al., 1990).

The generation of 8-hydroxydeoxyguanosine in calf thymus DNA treated with 2-phenylphenol, phenylhydroquinone, or phenylbenzoquinone, was studied *in vitro*. The content of 8-hydroxydeoxyguanosine residues was increased in DNA treated with phenylhydroquinone in a concentration-dependent manner, but phenylbenzoquinone had little effect, and 2-phenylphenol had no effect. The formation of 8-hydroxydeoxyguanosine by phenylhydroquinone was reduced by oxygen radical scavengers and accelerated by the addition of CuCl or $CuCl_2$. Hydroxyl radicals generated during oxidation of phenylhydroquinone thus contribute to the formation of 8-hydroxydeoxyguanosine in DNA, and copper ions facilitate the oxidative DNA damage. Copper ions greatly accelerated phenylhydroquinone-induced DNA cleavage *in vitro*, although they had no effect on cleavage without phenylhydroquinone. In contrast, DNA cleavage occurred with the addition of $FeCl_2$ in the absence and presence of phenylhydroquinone. The formation of 8-hydroxydeoxyguanosine in bladder DNA is likely to be one of a series of events in the carcinogenesis induced by 2-phenylphenol (Nagai et al., 1995).

The effect of the selective γ-glutamylcysteine synthetase inhibitor, buthionine sulfoximine, on the hepatotoxic and nephrotoxic potential of 2-phenylphenol and its metabolites was studied in

groups of four male Fischer 344/DuCrj rats. The animals were given an intraperitoneal injection of 0 or 900 mg/kg bw of buthionine sulfoximine and 1 h later received 2-phenylphenol, phenylhydroquinone, or phenylbenzoquinone at single oral doses of 0, 700, or 1400 mg/kg bw. The rats were killed 6 and 24 h later, and serum was collected for measurement of alanine and aspartate aminotransferase activities and urea nitrogen. The liver and kidneys were removed and weighed, and hepatic and renal glutathione were assayed.

2-Phenylphenol caused acute hepatocellular damage, as shown by necrotic centrilobular hepatocytes accompanied by increased serum aminotransferase activity. Pretreatment with buthionine sulfoximine potentiated the hepatic and renal toxicity of 2-phenylphenol, indicating that the liver and kidneys are its target organs of at high doses. 2-Phenylphenol depleted hepatic and renal glutathione by 6 h after administration, and this effect was enhanced by pretreatment with buthionine sulfoximine. Recovery of glutathione concentrations in both organs was slower in rats given 1400 mg/kg bw of 2-phenylphenol than in those given 700 mg/kg bw, suggesting that the hepatic and renal damage caused by this compound is associated with prolonged depletion of glutathione and that it acts indirectly on the liver. Within 24 h, 75% of the rats treated with phenylbenzoquinone at 1400 mg/kg bw had died. Administration of phenylbenzoquinone at 700 m/kg bw or phenylhydroquinone at 1400 mg/kg bw significantly increased aminotransferase activities. The activity of alanine aminotransferase in both groups was about twice that of rats given 1400 mg/kg bw 2-phenylphenol. A slight decrease in liver weight, nuclear pyknosis, eosinophilic degeneration of periportal hepatocytes, increased relative kidney weight, slight renal papillary necrosis, dilatation of renal tubules, and increased serum urea nitrogen concentration were observed at 700 mg/kg bw of phenylbenzoquinone. The relative weight of the kidneys was increased at 1400 mg/kg bw of phenylhydroquinone. These results indicate that phenylbenzoquinone is more toxic to liver and kidney than phenylhydroquinone (Nakagawa & Tayama, 1988).

In a study of the conjugation of 2-phenylphenol with glutathione in rat liver *in vitro* and *in vivo*, radiolabel derived from [^{14}C]2-phenylphenol bound irreversibly to hepatic microsomal macromolecules in an NADPH-generating system, and the binding was inhibited by cysteine and glutathione. When [^{14}C]2-phenylphenol and glutathione were incubated in a microsomal NADPH-generating system, the radiolabelled material derived from the aqueous phase of the incubation mixture was similar to a synthetic, water-soluble phenylhydroquinone–glutathione conjugate produced by a nonenzymic reaction between phenylbenzoquinone and glutathione. Phenylhydroquinone–glutathione was excreted as a minor conjugate in the bile after oral administration of 2-phenylphenol to rats at a dose of 1000 mg/kg bw. The cumulative biliary excretion of the conjugate over 6 h represented about 4% of the dose. The results show that a reactive intermediate of 2-phenylphenol can form adducts with glutathione to produce water-soluble conjugates. The reactive intermediate is probably phenylbenzoquinone derived from phenylhydroquinone. Since glutathione protects against cellular injury, the acute hepatic damage caused by high doses of 2-phenylphenol is probably associated with the formation of an active intermediate (phenylbenzoquinone) which depletes cellular glutathione (Nakagawa & Tayama, 1989).

The relationship between the metabolism and cytotoxicity of 2-phenylphenol was studied in isolated rat hepatocytes. Addition of high concentrations of 2-phenylphenol to the cells caused dose-dependent toxicity, with death at the highest dose of 1.0 mmol/L. Pretreatment of the hepatocytes with a non-toxic dose of 5 µmol/L of SKF-525A enhanced the cytotoxicity of 2-phenylphenol at 0.5–1.0 mmol/L and inhibited its metabolism. At lower concentrations (0.5 or 0.75 mmol/L), 2-phenylphenol was converted sequentially to phenylhydroquinone and then to its glutathione conjugate. The concentrations of both metabolites and especially of the conjugate, were very low in hepatocytes exposed to 2-phenylphenol at 1.0 mmol/L alone or with SKF-525A. The cytotoxicity induced by 2-phenylphenol at 0.5 mmol/L was enhanced by the addition of 1.25 mmol/L of diethylmaleate, which continuously depletes cellular glutathione. In contrast, the cytotoxicity induced by phenylhydroquinone at 0.5 mmol/L was significantly inhibited by addition to the hepatocytes of 5 mmol/L of dithiothreitol, cysteine, *N*-acetyl-L-cysteine, or ascorbic acid. Loss of glutathione, protein thiols, and ATP was also prevented. These results indicate that the acute cytotoxicity of 2-phenylphenol at 1.0 mmol/L is a direct action and that

prolonged depletion of cellular glutathione enhances the cytotoxicity of low concentrations of 2-phenylphenol metabolites. The cytotoxicity of phenylhydroquinone is prevented significantly by addition of cysteine, glutathione, or ascorbic acid (Nakagawa et al., 1992).

Groups of 22 male CDF (Fischer 344)/BR rats were given diets containing 2phenylphenol (purity, 99.5%) to provide concentrations of 0, 800, 4000, 8000, or 12 500 ppm, equal to 0, 56, 280, 560, and 920 mg/kg bw per day, for 13 weeks. During weeks 12–13 and 13–14 of the study, urine was collected for determination of metabolites and urinary characteristics, respectively. In addition, urinary bladders were collected from 12 animals per group during week 14 for analysisof the urothelium by ^{32}P-postlabelling, while histopathological evaluation of 10 animals group included determination of a labelling index and light and scanning electron microscopy. The body-weight gain was reduced by about 10% at 8000 and 12 500 ppm, but food intake was unaffected at all doses tested. Histological examination showed simple hyperplasia of the urothelium at concentrations of 8000 and 12 500 ppm with significant changes in the bladder. The glucuronide and sulfate conjugates of 2-phenylphenol and the hydroxylated metabolite phenylhydroquinone were the major urinary metabolites, although the major conjugate at all doses was the sulfate. Minute levels of free 2phenylphenol and phenylhydroquinone were found at all doses, free phenylhydroquinone comprising 0.6–1.5% of the total metabolites measured. An increase in the labelling index of the bladder epithelium was observed at 8000 and 12 500 ppm. ^{32}P-Postlabelled urothelial DNA showed no evidence of formation of 2-phenylphenol–DNA adducts.

The authors concluded that a hyperplastic response of the urinary bladder epithelium occurs after exposure to 2-phenylphenol at 8000 or 12 500 ppm, which are unequivocal carcinogenic doses for the bladder of male rats, which is due to mild cytotoxicity with consequent regenerative hyperplasia. The increased mitotic activity (labelling index), the presence of very small amounts of free phenylhydroquinone in the urine, and the absence of DNA adducts in the bladder epithelium further suggest that the bladder carcinogenesis in male rats exposed to 2-phenylphenol is probably mediated by an indirect, dose-dependent cytotoxic effect on the bladder epithelium leading to regenerative hyperplasia and subsequent tumorigenesis of epigenetic origin, rather than to direct metabolic activation of 2-phenylphenol to reactive metabolites capable of forming 2-phenylphenol–DNA adducts (Christenson et al., 1996a).

Groups of 20–30 male CDF (Fischer344)/BR rats were given diets containing 2-phenylphenol (purity, 99.9%) at concentrations of 0, 1000, 4000, or 12 500 ppm, equal to 0, 54, 220, and 680 mg/kg bw per day, for 13 weeks. Animals from the control and high-dose groups were allowed to recover for 4 weeks. Urine was collected for chemical and and electron microscopic evaluation at various times, and urinary bladders were collected from animals in the recovery groups during weeks 4, 13, and 17 for histological evaluations which included determination of a labelling indexes and light and electron microscopy.

Body-weight gain was reduced only in rats at 12 500 ppm, and food intake was unaffected at all doses. Weekly clinical examinations showed an increased incidence of urine staining at 4000 and 12 500 ppm. No unusual precipitate or crystal was found in the urinary sediment of treated animals. Urothelial hyperplasia was observed only after 13 weeks at 12 500 ppm, and the effect was reversed by 4 weeks on control diet. After 4 and 13 weeks of exposure to 2-phenylphenol, necrotic foci were observed in the bladders of rats at 12 500 ppm, and at 13 weeks the bladders also showed evidence of regenerative hyperplasia. Increased labelling indexes were observed in the bladders of animals at the high dose at 4 and 13 weeks, but the index had returned to control values after 4 weeks' recovery, confirming the reversibility of the proliferative changes in the urothelium. The results of this study suggest that 2-phenylphenol acts by a mechanism involving a cytotoxic action on the urothelium leading to the formation of regenerative, reversible hyperplasia. The origin of the cytotoxicity remains unclear, however, as no evidence was found of either abnormal crystalluria or a calcium phosphate-containing amorphous precipitate (Christenson et al. 1996b).

The relative importance of bladder distension, urinary pH, and Na^+ concentration in the induction of cell proliferation in the bladder epithelium of rats fed various sodium salts was investigated. In male rats fed a diet containing 5% $NaHCO_3$, the bladder epithelium showed an increased number of replicating cells, distension, increased urinary pH, and a high urinary Na^+

concentration. Cell proliferation also occurred when the bladders were subjected to distension *in vivo* by mechanical (female) or physiological (male) means. Inclusion of $CaCO_3$ in the diet increased the urinary pH without altering other factors and did not induce cell proliferation, but proliferation was increased when $CaCO_3$ was combined with the mechanical or physiological treatment. Thus, high urinary pH was of secondary importance to bladder distension as a causative factor but acted to enhance cell proliferation when distension occurred. Similar findings were obtained with regard to the Na^+ concentration. The authors concluded that bladder distension is a prerequisite for proliferation of epithelial cells in the bladders of rats fed diets containing high concentrations of sodium salts and that changes in urinary pH and Na^+ concentration also determine the degree of proliferation (Shioya et al., 1994).

3. Observations in humans

In one of the earliest studies on the toxicity of 2-phenylphenol, skin irritation and sensitization due to exposure to this compound and its sodium salt were evaluated in 100 male and 100 female, unselected persons. A patch impregnated with the test material was placed in direct contact with the skin of the back of each person, covered with an impervious film, and taped securely in place. The first patch was kept in constant contact with the skin for 5 days, at which time the patch was removed and the reaction noted. A second patch was applied in the same way 3 weeks after removal of the first patch and was kept in direct contact with the skin for 48 h. Each subject was examined immediately and again 3 and 8 days after removal of the second patch. 2-Phenylphenol as a 5.0% solution in sesame oil did not cause primary irritation or sensitization, but sodium 2-phenylphenol was significantly irritating when applied as a 5% or a 1% aqueous solution. A 0.5% solution caused very slight, simple irritation, whereas a 0.1% solution produced no irritation and no sensitization (Hodge et al., 1952).

Comments

After oral administration to mice and rats, 2-phenylphenol and its sodium salt are rapidly and extensively absorbed (95%) and distributed. Excretion is also rapid in these species, being almost complete within 48 h, and occurs mainly in urine (about 90%) and in faeces (about 5%). Little radiolabel (< 1%) is retained in organs and tissues, including the urinary bladder. After dermal application of 2-phenylphenol to humans, about 43% of the applied dose was absorbed through the skin and about 58% was recovered in skin rinse and the protective enclosure. Most of the absorbed radiolabel was recovered in urine (99%, and only 1% was recovered in faeces. The absorption half-time was 10 h, and the elimination half-time was 0.8 h. The rapid excretion of the radiolabel into urine indicates that 2-phenylphenol is unlikely to accumulate in humans exposed repeatedly. The metabolic profiles of both compounds were similar in mice, rats, and humans at the various doses tested. The main metabolic pathways are conjugation of 2-phenylphenol or hydroxylation at the 5 position of the phenol ring, followed by conjugation with glucuronide or sulfate. The parent compound was detected in only very small amounts (0.4%) in urine. The metabolic profile in plants raised no toxicological concern, since about 90% of the residue found in oranges and pears is 2-phenylphenol or its conjugates.

2-Phenylphenol and its sodium salt have low acute toxicity in mice and rats treated orally, the LD_{50} values ranging from 600 to 3500 mg/kg bw. Neither 2-phenylphenol nor its sodium salt has been classified by WHO for acute toxicity.

2-Phenylphenol and its sodium salt caused severe dermal irritation in rabbits, and the sodium salt caused severe dermal irritation in humans. 2-Phenylphenol irritated the eye of rabbits, whereas the sodium salt caused only moderate ocular irritation. Neither substance caused delayed contact hypersensitivity in guinea-pigs or humans.

In medium- and long-term tests for toxicity, the urinary bladder was regarded as the main toxicological target organ of both 2-phenylphenol and its sodium salt in male and female rats. At doses of 200 mg/kg bw per day and above, hyperplasia, papillomas, and transitional-cell carcinomas were seen with both compounds in male rats. Increased mitosis was observed in the bladder epithelium three days after the start of dosing, and thickening, i.e. simple hyperplasia, was

seen at 14 days. In female rats, hyperplasia and papillomas were observed, but to a far lower degree than in males. In male and female mice, the liver was the primary target organ. Increased relative liver weights and an increased incidence of hepatocellular adenomas were seen with 2-phenylphenol at doses of 500 mg/kg bw per day and above. Reduced body-weight gain was a common finding in mice and rats. In 90-day studies, the NOAELs for 2-phenylphenol were 6300 ppm, equal to 760 mg/kg bw per day, in rats and 300 mg/kg bw per day (the highest dose tested for up to 1 year) in dogs. The NOAEL for the sodium salt was 5000 ppm, equivalent to 550 mg/kg bw per day, in mice and 2500 ppm, equal to 180 mg/kg bw per day, in rats. In a 1-year study of toxicity, the NOAEL for 2-phenylphenol was 800 ppm, equal to 39 mg/kg bw per day, in rats. In 2-year studies of carcinogenicity, the NOAEL for 2-phenylphenol was 250 mg/kg bw per day in mice and 800 ppm, equal to 39 mg/kg bw per day, in rats. In 2-year carcinogenicity studies with the sodium salt, the NOAEL for carcinogenicity was 20 000 ppm, equal to 3000 mg/kg bw per day, in mice and 2500 ppm, equivalent to 95 mg/kg bw per day, in rats. The Meeting concluded that both 2-phenylphenol and its sodium salt are carcinogenic in male rats and that 2-phenylphenol is carcinogenic in male mice.

2-Phenylphenol has been more extensively tested for genotoxic activity than its sodium salt. Within that limitation, the results for the two compounds were similar. Data regarding covalent binding to DNA in the urinary bladder of rats dosed with either compound were conflicting. 2-Phenylphenol induced chromosomal aberrations in cultured mammalian cells, but negative results were obtained *in vivo*. The Meeting concluded that there are unresolved questions about the genotoxic potential of 2-phenylphenol.

Several studies have been conducted to elucidate the mechanism of the carcinogenic action of 2-phenylphenol and its sodium salt on the male rat urinary bladder, since neither compound has a carcinogenic effect on the urinary bladder of female rats or in mice, guinea-pigs, or hamsters of either sex. No clear mechanisms have been found, although raising the urinary pH or sodium concentration has a promoting effect. There was some evidence from studies with the sodium salt that initial irritation followed by hyperplasia might be involved in the bladder carcinogenicity in male rats. In addition, ^{32}P-postlabelling showed binding of 2-phenylphenol and its sodium salt to DNA in the male rat urinary bladder in some but not in other studies. The genotoxicity of the metabolites phenylhydroquinone and dihydroxybiphenyl appears to be similar to that of the parent molecules.

The Meeting concluded that the urinary bladder tumours observed in male rats and the liver tumours observed in male mice exposed to 2-phenylphenol are threshold phenomena that are species- and sex-specific, and that 2-phenylphenol is therefore unlikely to represent a carcinogenic risk to humans. In coming to this conclusion, the Meeting was aware that a working group convened by IARC had classified 2-phenylphenol, sodium salt, in Group 2B (possibly carcinogenic to humans) and 2-phenylphenol in Group 3 (not classifiable as to its carcinogenicity to humans). The Meeting noted, however, that the IARC classification is based on hazard identification, not on risk assessment, and is furthermore limited to published literature, with the exclusion of unpublished studies on toxicity and carcinogenicity.

In two two-generation studies of reproductive toxicity in rats, 2-phenylphenol had no reproductive toxicity, even at 460 mg/kg bw per day, the highest dose tested. The overall NOAEL for carcinogenicity was 92 mg/kg bw per day, since urinary bladder tumours were found in male rats at doses of 120 mg/bw per day and above.

In a study of developmental toxicity in mice with 2-phenylphenol and its sodium salt, the NOAELs for 2-phenylphenol were below 1500 mg/kg bw per day (lowest dose tested) for maternal toxicity and fetotoxicity and 2100 mg/kg bw per day (highest dose tested) for teratogenicity. The NOAELs for the sodium salt were below 100 mg/kg bw per day (lowest dose tested) for maternal toxicity, 100 mg/kg bw per day for fetotoxicity, and 400 mg/kg bw per day (highest dose tested) for teratogenicity. In two studies of developmental toxicity in rats, the overall NOAELs for 2-phenylphenol were 150 mg/kg bw per day for maternal toxicity, 300 mg/kg bw per day for fetotoxicity, and 700 mg/kg bw per day (highest dose tested) for teratogenicity. In two studies of developmental toxicity in rabbits, the overall NOAELs for 2-phenylphenol were 100 mg/kg bw per day for maternal toxicity, 500 mg/kg bw per day for fetotoxicity, and 750 mg/kg bw per day (highest dose tested) for teratogenicity.

The Meeting established an ADI of 0–0.4 mg/kg bw for 2-phenylphenol, on the basis of the NOAEL of 39 mg/kg per day in the 2-year study of toxicity (based on decreased body-weight gain and hyperplasia of the urinary bladder) and carcinogenicity of the urinary bladder in male rats and a safety factor of 100.

The Meeting determined that it was unnecessary to establish an acute reference dose because of the low acute toxicity of 2-phenylphenol.

Toxicological Evaluation

Levels of 2-phenylphenol that cause no toxic effect

Mouse: < 250 mg/kg bw per day for carcinogenicity (lowest dose tested; 2-year study of toxicity and carcinogenicity)
< 1500 mg/kg bw per day (lowest dose tested; study of developmental toxicity; maternal toxicity)
2100 mg/kg bw per day (highest dose tested; study of developmental toxicity; not teratogenic)

Rat: 800 ppm, equal to 39 mg/kg bw per day (2-year study of toxicity and carcinogenicity)
460 mg/kg bw per day (two-generation study of reproductive toxicity; no reproductive toxicity; highest dose tested)
92 mg/kg bw per day (two-generation study of reproductive toxicity; carcinogenicity)
150 mg/kg bw per day (study of developmental toxicity; maternal toxicity)
300 mg/kg bw per day (study of developmental toxicity; developmental toxicity)
700 mg/kg bw per day (study of developmental toxicity; teratogenicity)

Rabbit: 100 mg/kg bw per day (two studies of developmental toxicity; maternal toxicity)
500 mg/kg bw per day (two studies of developmental toxicity; fetotoxicity)
750 mg/kg bw per day (two studies of developmental toxicity; teratogenicity)

Dog: 750 mg/bw per day (highest dose tested; 1-year study of toxicity)

Estimate of acceptable daily intake for humans

0–0.4 mg/kg bw

Estimate of acute reference dose

Unnecessary

Studies that would provide information useful for continued evaluation of the compound

1. Mechanistic studies on urinary bladder tumours in male rats
2. Further observations in humans

Toxicological end-points relevant for estimating guidance values for dietary and non-dietary exposure to 2-phenylphenol (unless otherwise specified)

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Rapid (24 h) and complete (95–100%), mice and rats
Dermal absorption	Rapid and well absorbed (43%), humans
Distribution	Small concentrations (< 1%) in tissues, mice and rats
Potential for accumulation	No accumulation, mice, rats, and humans

Toxicological end-points (contd)

Rate and extent of excretion	Rapid and complete (95–100%), mice, rats, and humans
Metabolism in animals	Glucuronide and sulfate of 2-phenylphenol and phenylhydroquinone, mice and rats
Toxicologically significant compounds (animals, plants and environment)	2-Phenylphenol
Acute toxicity	
Rat, LD_{50}, oral	2800 mg/kg bw
Rabbit, LD_{50}, dermal	> 5000 mg/kg bw
Rabbit, LC_{50}, inhalation (4 h)	> 36 mg/m³ air (aerosol)
Dermal irritation	2-Phenylphenol and its sodium salt: severe dermal irritation, rabbits
Ocular irritation	2-Phenylphenol: ocular irritation, rabbits Sodium salt: slight ocular irritation, rabbits
Dermal sensitization	2-Phenylphenol and its sodium salt: no dermal sensitization, guinea-pigs and humans
Short-term toxicity	
Target/critical effect	Body-weight decrease, mice and rats, and urinary bladder tumours, male rats
Lowest relevant oral NOAEL	300 mg/kg bw per day, dogs
Lowest relevant dermal NOAEL	No NOAEL, 1000 mg/kg bw per day, highest dose tested, rats
Lowest relevant inhalation NOAEL	Not investigated
Genotoxicity	Unresolved questions
Long-term toxicity and carcinogenicity	
Target/critical effect	Urinary bladder, male rats Liver, male and female mice
Lowest relevant NOAEL	39 mg/kg bw per day, male rats
Carcinogenicity	Urinary bladder tumours, male rats Liver tumours, male and female mice
Reproductive toxicity	
Reproductive target/critical effect	No reproductive toxicity, rats
Lowest relevant reproductive NOAEL	460 mg/kg bw per day, highest dose tested, rats
Developmental target/critical effect	Developmental toxicity at maternally toxic doses, mice
Lowest relevant developmental NOAEL	300 mg/kg bw per day, rabbits
Neurotoxicity/Delayed neurotoxicity	No evidence of developmental neurobehavioural toxicity in rats. No evidence of neurotoxicity or neuropathology in medium- and long-term studies, mice, rats, dogs, or in developmental toxicity studies, mice, rats, and rabbits
Other toxicological studies	
Medical data	Dermal irritation with the sodium salt, not with 2-phenylphenol

Summary	*Value*	*Study*	*Safety factor*
ADI	0–0.4 mg/kg bw	Long-term study of toxicity and carcinogenicity, rat	100
Acute reference dose	Unnecessary		

References

Bartels, M.J. & McNett, D.A. (1996) Quantitation of orthophenylphenol metabolites in rat urine samples from a ^{32}P-postlabeling study. Unpublished report No. HET K-001024-062 from Dow Chemical Co., Midland, Michigan, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Bartels, M.J., Brzak, K.A., McNett, D.A. & Shabrang, S.N. (1997) Ortho-phenylphenol (OPP): Limited metabolism study in human. Unpublished report No. HET K-001024-059 from Dow Chemical Company, Midland, Michigan, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Bartels, M.J., McNett, D.A., Timchalk, C., Mendrala, A.L., Christenson, W.R., Sangha, G.K., Brzak, K.A. & Shabrang, S.N. (1998) Comparative metabolism of *ortho*phenylphenol in mouse, rat and man. *Xenobiotica*, **28**, 579–594.

Berdasco, N.M. (1991) Orthophenylphenol: Dermal sensitization potential in the Hartley albino guinea pig. Unpublished report No. K-001024-048 from Dow Chemical Co., Midland, Michigan, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Carreon, R.E. & New M.A. (1981) Dowicide*1: Acute percutaneous absorption potential. Unpublished report No. HET K-1024-(37) from Dow Chemical Co., Midland, Michigan, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Christenson, W.R., Wahle, B.S., Bartels, M.J. & Cohen, S.M. (1996a) Technical grade *ortho*phenylphenol: A ^{32}P-postlabeling study to examine the potential for the formation of DNA adducts in the urinary bladder of the male rat. Unpublished report No. 94-972-AV from Dow Chemical Co., Midland, Michigan, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Christenson, W.R., Wahle, B.S. & Cohen, S.M. (1996b) Technical grade *ortho*-phenylphenol: A special subchronic dietary study to examine the mechanism of urinary bladder carcinogenesis in the male rat. Unpublished report No. 92-972-MS from Bayer Corporation, Agriculture Division, Toxicology, Stilwell, Kansas, and University of Nebraska Medical Center, Department of Pathology & Micropathology, Omaha, Nebraska, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Cosse, P.F., Stebbins, K.E., Stott, W.T., Johnson, K.A. & Atkin, L. (1990) Orthophenylphenol: Palatability/ probe, four-week and one-year oral toxicity studies in beagle dogs. Unpublished report No. K-001024-039 from Dow Chemical Co., Midland, Michigan , USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Davies, R.E. & Liggett, M.P. (1973) Irritant effects of agricultural disinfectant C2 on rabbit eye mucosa. Unpublished report No. 2215/169D/73 from Huntingdon Research Centre, England. Submitted to WHO by Leng Associates, Midland, Michigan, USA,

Eigenberg, D.A. (1990) Two-generation dietary reproduction study in rats using orthophenylphenol. Unpublished revised report No 85-671-02 from Mobay Corporation Health, Environment, Safety & Plant Management, Corporate Toxicology Department, South Metcalf, Stilwell, Kansas, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Eigenberg, D.A. (1995) A two-generation dietary reproduction study in Sprague Dawley rats using technical grade ortho-phenylphenol. Unpublished report No. 93-672-VX from Bayer Corporation Agriculture Division, Toxicology, South Metcalf, Stilwell, Kansas, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Fujii, T. & Hiraga, K. (1985) Carcinogenicity testing of sodium orthophenylphenate in F344 rats. *J. Saitama Med. School*, **12**, 277–287.

Fujii, T., Nakamura, K. & Hiraga, K. (1987) Effects of pH on the carcinogenicity of *o*phenylphenol and sodium *o*-phenylphenate in the rat urinary bladder. *Food Chem. Toxicol.*, **25**, 359–362.

Fukushima, S., Kurata, Y., Shibata, M., Ikawa, E. & Ito, N. (1983) Promoting effect of sodium *o*-phenylphenate and *o*-phenylphenol on two-stage urinary bladder carcinogenesis in rats. *Jpn. J. Cancer Res. (Gann)*, **74**, 625–632.

Fukushima, S., Inoue, T., Uwagawa, S., Shibata, M. & Ito N. (1989) Co-carcinogenic effects of $NaHCO_3$ on *o*-phenylphenol-induced rat bladder carcinogenesis. *Carcinogenesis*, **10**, 1635–1640.

Gilbert, K.S. (1994a) Dowicide® 1 antimicrobial: Primary dermal irritation study in New Zealand white rabbits. Unpublished report No. K-001024-057B from Toxicological Research Laboratory, Health & Environmental Sciences, Dow Chemical Co., Midland, Michigan, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Gilbert, K.S. (1994b) Dowicide® 1 antimicrobial: Dermal sensitization potential in the Hartley albino guinea pig. Unpublished report No. K-001024-57E from Toxicological Research Laboratory, Health & Environmental Sciences, Dow Chemical Co., Midland, Michigan, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Gilbert, K.S. (1994c) Dowicide® A antimicrobial: Dermal sensitization potential in the Hartley albino guinea pig. Unpublished report No. K-001025-014E from Toxicological Research Laboratory, Health & Environmental Sciences, Dow Chemical Co., Midland, Michigan, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Gilbert, K.S. & Crissman, J.W. (1994) Dowicide® 1 antimicrobial: Acute oral toxicity study in Fischer 344 rats. Unpublished report no. K-001024-057A from Toxicological Research Laboratory, Health & Environmental Sciences, Dow Chemical Co., Midland, Michigan, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Gilbert K.S. & Stebbins, K. (1994) Dowicide® A antimicrobial: Acute oral toxicity study in Fischer 344 rats. Unpublished report No. K-001025-014A from Toxicological Research Laboratory, Health & Environmental Sciences, Dow Chemical Co., Midland, Michigan, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Hagiwara, A., Shibata, M., Hirose, M., Fukushima, S. & Ito, N. (1984) Long-term toxicity and carcinogenicity study of sodium *o*-phenylphenate in B6C3F1 mice. *Food Chem. Toxicol.*, **22**, 809–814.

Hasegawa, R., Takahashi, S., Asamoto, M., Shirai, T. & Fukushima, S. (1990a) Species differences in sodium *o*-phenylphenate induction of urinary bladder lesions. *Cancer Lett.*, **50**, 87–91.

Hasegawa, R., Furukawa, F., Toyoda K., Sato, H., Takahashi, M. & Hayashi, Y. (1990b) Urothelial damage and tumor initiation by urinary metabolites of sodium *o*-phenylphenate in the urinary bladder of female rats. *Jpn. J. Cancer Res.*, **81**, 483–488.

Hasegawa, R., Fukuoka, M., Takahashi, T., Yamamoto, A., Yamaguchi, S., Shibata, M.A., Tanaka, A. & Fukushima, S. (1991) Sex differences in *o*-phenylphenol and sodium *o*phenylphenate rat urinary bladder carcinogenesis: Urinary metabolites and electrolytes under conditions of aciduria and alkalinuria. *Jpn. J. Cancer Res.*, **82**, 657–664.

Hiraga, K. (1983a) Short report of a 91-week feeding study of orthophenylphenol (OPP) in male rats. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Hiraga, K. (1983b) Carcinogenicity testing of sodium orthophenylphenate in F-344/DuCrj rats. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Hiraga, K. & Fujii, T. (1981) Induction of tumours of the urinary system in F344 rats by dietary administration of sodium *o*-phenylphenate. *Food Cosmet. Toxicol.*, **19**, 303–310.

Hiraga, K. & Fujii, T. (1984) Induction of tumours of the urinary bladder in F344 rats by dietary administration of o-phenylphenol. *Food Cosmet. Toxicol.*, **22**, 865–870.

Hodge, H.C., Maynard, E.A., Blanchet, H.J., Jr, Spencer, H.C. & Rowe, V.K. (1952) Toxicological studies of orthophenylphenol (Dowicide 1). *J. Pharmacol. Exp. Ther.*, **104**, 202–210.

Honma, Y., Kakizoe, T., Komatsu, H., Niijima, T. & Sugimura, T. (1983) Increased agglutinability of bladder epithelial cells by concanavalin A in rats fed several biphenyl derivatives. *J. Cancer Res. Clin. Oncol.*, **106**, 176–178.

Horvath, E., Levay, G. Pongracz, K. & Bodell, W.J. (1992) Peroxidative activation of *o*phenylhydroquinone leads to the formation of DNA adducts in HL-60 cells. *Carcinogenesis*, **13**, 1937–1939.

IARC (1987) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, International Agency for Research on Cancer, pp. 37–41.

IARC (1999) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 73, *Miscellaneous Pesticides*, Lyon, International Agency for Research on Cancer, pp. 451–480.

Iguchi, S., Takahashi, H., Fujii, T., Fukumori, N., Mikuriya, H., Tada, Y., Yuzawa, K. & Hiraga, K. (1984) Subchronic toxicity of *o*-phenylphenol (OPP) by food administration to rats. *Ann. Rep. Tokyo Metr. Res. Lab. Public Health*, **35**, 407–415.

Iguchi, S., Tayama, K. & Hiraga, K. (1979) Subacute toxicity of sodium *o*-phenylphenate by food administration to rats. *Ann. Rep. Tokyo Metropolitan Res. Lab. Public Health*, **30**, 67–79.

Inoue, T. (1993) Pathological studies on rat urinary bladder carcinogenicity of *o*phenylphenol and its sodium salt. *J. Nagoya City Univ. Medical Assoc. (Nagoya Shiritsu Daigaku Igakkai Zasshi)*, **44**, 463–477.

Inoue, S., Yamamoto, K. & Kawanishi, S. (1990) DNA damage induced by metabolites of *o*phenylphenol in the presence of copper (II) ion. *Chem. Res. Toxicol.*, **3**, 144–149.

Ishidate, M., Jr, Yoshikawa, K. & Sofuni, T. (1983) Mutagenicity tests on OPP, OPPsodium salt, and their possible metabolites. Unpublished report from Division of Mutagenesis, Biological Safety Research Center, National Institute of Hygienic Sciences, Tokyo, Japan. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Ito, N. (1983a) Long-term toxicity and carcinogenicity study of sodium o-phenyl-phenate in B6C3F1 mice. Unpublished report from First Department of Pathology, Nagoya City University Medical School, Nagoya, Japan. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Ito, N. (1983b) Pathological analysis of the carcinogenicity of sodium o-phenyl-phenate and *o*-phenylphenol. Unpublished report from First Department of Pathology, Nagoya City University Medical School, Nagoya, Japan. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

John, J.A., Murray, F.J., Rao, K.S. & Schwetz B.A. (1981) Teratological evaluation of orthophenylphenol in rats. *Fundam. Appl. Toxicol.*, **1**, 282–285.

Kaneda, M., Teramoto, S., Shingu, A. & Shirasu, Y. (1978) Teratogenicity and dominantlethal studies with *o*-phenylphenol. *J. Pestic. Sci.*, **3**, 365–370.

Kolachana, P., Subrahmanyam, V.V., Eastmond, D.A. & Smith, M.T. (1991) Metabolism of phenyhydroquinone by prostaglandin (H) synthase: Possible implications in *o*phenylphenol carcinogenesis. *Carcinogenesis*, 12, 145–149.

Kwok, E.S.C. & Eastmond, D.A. (1997) Effects of pH on nonenzymatic oxidation of phenylhydroquinone: Potential role in urinary bladder carcinogenesis induced by *o*phenylphenol in Fischer 344 rats. *Chem. Res. Toxicol.*, 10, 742–749.

Lambert, A.C. & Eastmond, D.A. (1994) Genotoxic effects of the *o*-phenylphenol metabolites phenylhydroquinone and phenylbenzoquinone in V79 cells. *Mutat. Res.*, 322, 243–256.

Landry, T.D., Stebbins, K.E. & Battjes, J.E. (1992) Ortho-phenylphenol: Acute aerosol inhalation toxicity study in Fischer 344 rats. Unpublished report No. K-001024-049 from Toxicological Research Laboratory, Health & Environmental Sciences, Dow Chemical Co., Midland, Michigan, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Luster, M. (1986) *NTP Technical Report on the Toxicology and Carcinogenesis Studies of ortho-Phenylphenol (CAS No. 90-43-7) Alone and with 7,12-Dimethylbenz(a)anthracene (CAS No. 57-97-6) in Swiss CD-1 Mice (Dermal Studies)* (NIH publication No. 86-2557, NTP TR 301), US Department of Health and Human Services, National Toxicology Program, Research Triangle Park, North Carolina, USA.

Luster, M.I., Dean, J.H., Boorman, G.A., Archer, D.L., Lauer, L., Lawson, L.D., Moore, J.A. & Wilson, R.E. (1981) The effects of orthophenylphenol, tris(2,3dichloropropyl)phosphate and cyclophosphamide on the immune system and host susceptibility of mice following subchronic exposure. *Toxicol. Appl. Pharmacol.*, 58, 252–261.

McNett, D.A., Timchalk, C. & Mendrala, A.L. (1997) Ortho-phenylphenol (OPP): Metabolism of ^{14}C-labelled OPP in B6C3F$_1$ mice and Fischer 344 rats. Unpublished report No. HET K-001024-060 from Toxicological Research Laboratory, Health & Environmental Sciences, Dow Chemical Co., Midland, Michigan, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Morimoto, K., Sato, M., Fukuoka, M., Hasegawa, R., Takahashi, T., Tsuchiya, T., Tanaka, A., Takahashi, A. & Hayashi, Y. (1989) Correlation between the DNA damage in urinary bladder epithelium and the urinary 2-phenyl-1,4-benzoquinone levels from F344 rats fed sodium *o*-phenylphenate in the diet. *Carcinogenesis*, 10, 1823–1827.

Nagai, F. & Nakao, T. (1984) Changes in enzyme activities in the urine and tissues of rats fed sodium *o*-phenylphenate. *Food Chem. Toxicol.*, 22, 361–364.

Nagai, F., Ushiyama, K., Satho, K. & Kano, I. (1990) DNA cleavage by phenylhydroquinone: The major metabolite of a fungicide o-phenylphenhol. *Chem.-Biol. Interactions*, 76, 163–179.

Nagai, F., Ushiyama, K., Satoh, K., Kasai, H. & Kano, I. (1995) Formation of 8hydroxyguanosine in calf thymus DNA treated *in vitro* with phenylhydroquinone, the major metabolite of *o*-phenylphenol. *Carcinogenesis*, 16, 837–840.

Nakagawa, Y. & Tayama, K. (1988) Effect of buthionine sulfoximine on orthophenylphenolinduced hepato- and nephrotoxic potential in male rats. *Arch. Toxicol.*, 62, 452–457.

Nakagawa, Y. & Tayama, S. (1989) Formation of ortho-phenylphenol ghutathione conjugates in the rat liver. *Xenobiotica*, 19, 499–507.

Nakagawa, A., Nakao, T. & Nakao, M. (1984) Altered levels of cyclic nucleotides in F344 rats fed sodium *o*-phenylphenate. *Food Chem. Toxicol.*, 22, 217–221.

Nakagawa Y., Tayama, S., Moore, G.A. & Moldéus, P. (1992) Relationship between metabolism and cytotoxicity of *ortho*-phenylphenol in isolated rat hepatocytes. *Biochem. Pharmacol.*, 43, 1431–1437.

Nakao, T., Kabashima, U.J., Nagai, F., Nakagawa, A., Ohno, T., Ichikawa, H., Kabayashi, H. & Hiraga, K. (1983) The metabolic profile of sodium *o*-phenylphenate after subchronic oral administration to rats. *Food Chem. Toxicol.*, 21, 325–329.

National Toxicology Program (1986) *Toxicology and Carcinogenesis Studies of orthoPhenylphenol (CAS No. 90-43-7) Alone and with 7,12-Dimethylbenz(a)anthracene (CAS No. 57-97-6) in Swiss CD-1 Mice (Dermal Studies)* (NTP Technical Report No. 301, NIH publication No. 86-2557), US Department of Health and Human Services, Research Triangle Park, North Carolina, USA.

Norris, J..M. (1971) Eye irritation test conducted on Dowicide l preservative. Unpublished report from Chemical Biology Research, Dow Chemical Co., Midland, Michigan, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Oehme, F.W. & Smith, T.H.F. (1972) The metabolism and urinary excretion of *o*phenylphenol in dogs and cats. *Toxicol. Appl. Pharmacol.*, 22, 292.

Ogata, A., Ando, H., Kubo, Y. & Hiraga, K. (1978) Teratological tests of ortho-phenylphenol and its sodium salt in mice. *Ann. Rep. Tokyo Metr. Res. Lab. Public Health*, 29, 89–96.

Ogata, A., Ando, H., Kubo, Y. & Hiraga, K. (1979) Acute oral toxicity of sodiumphenylphenate (OPP-Na) in mice. *Ann. Rep. Tokyo Metr. Res. Lab. ublic Health*, 30, 54.

Pathak, D.N. & Roy, D. (1992) Examination of microsomal cytochrome P450-catalyzed *in vitro* activation of *o*-phenylphenol to DNA binding metabolite(s) by ^{32}P-postlabeling technique. *Carcinogenesis*, 13, 1593–1597.

Pathak, D.N. & Roy, D. (1993) In vivo genotoxicity of sodium *ortho*-phenylphenol: phenylbenzoquinone is one of the DNA-binding metabolite(s) of sodium *ortho*phenylphenol. *Mutat. Res.*, **286**, 309–319.

Quast, J.F. & McGuirk, R.J. (1995) *ortho*-Phenylphenol: Two-year dietary chronic toxicity/oncogenicity study in B6C3F1 mice. Unpublished report No. K-001024-047 from Dow Chemical Co., Midland, Michigan, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Reitz, R.H., Fox, T.R., Quast, J.F., Hermann, E.A. & Watanabe, P.G. (1983) Molecular mechanisms involved in the toxicity of orthophenylphenol and its sodium salt. *Chem.-Biol. Interactions*, **43**, 99–119.

Reitz, R.H., Fox, T.R., Quast, J.F., Hermann, E.A. & Watanabe, P.G. (1984) Biochemical factors involved in the effects of orthophenylphenol (OPP) and sodium orthophenylphenate (SOPP) on the urinary tract of male F344 rats. *Toxicol. Appl. Pharmacol.*, 73, 345–349.

Roy, D. (1990) Cytochrome P-450 catalyzed redox cycling of orthopenylphenol. *Biochem. Int.*, **22**, 849–857.

Sato, M., Tanaka, A., Tsuchiya, T., Yamaha, T., Nakaura, S. & Tanaka, S. (1988) Excretion, distribution and metabolic fate of sodium *o*-phenylphenate and *o*- phenylphenol in the rat. *J. Food Hyg. Soc. Jpn*, **29**, 7–12.

Savides, M.C. & Oehme, F.W. (1980) Urinary metabolism of orally administered *ortho*phenyl phenol in dogs and cats. *Toxicology*, **17**, 355–363.

Selim, S.A. (1996) A single dose open label study to investigate the absorption and excretion of $^{14}C/^{13}C$-labeled orthophenylphenol formulation after dermal application to healthy volunteers. Unpublished report No. P0995002 from Pharma Bio-Research Clinics BV, Assen, Netherlands. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Shibata, M.-A., Hagiwara, A., Tamano, S., Fukushima, S. & Ito, N. (1985) Subchronic toxicity study of sodium *o*-phenylphenate in mice. *Toxicol. Lett.*, **25**, 239–246.

Shibata, M.-A., Tanaka, H., Yamada, M., Tamano, S. & Fukushima, S. (1989a) Proliferative response of renal pelvic epithelium in male rats to oral administration of *ortho*phenylphenol, sodium *ortho*-phenylphenate and diphenyl. *Cancer Lett.*, **48**, 19–28.

Shibata, M.-A., Tamano, S., Kurata, Y., Hagiwara, A. & Fukushima, S. (1989b) Participation of urinary Na^+, K^+, pH, and L-ascorbic acid in the proliferative response of the bladder epithelium after the oral administration of various salts and/or ascorbic acid to rats. *Food Chem. Toxicol.*, **27**, 403–413.

Shioya, S., Nagami-Oguihara, R., Oguihara, S., Kimura, T., Imaida, K. & Fukushima, S. (1994) Roles of bladder distension, urinary pH and urinary sodium ion concentration in cell proliferation of urinary bladder epithelium in rats ingesting sodium salts. *Food Chem. Toxicol.*, **32**, 165–171.

Shirasu, Y., Moriya, M., Kato, K., Tezuka, H., Hemmi, R., Shingu, A., Kaneda, M. & Teramoto, S. (1978) Mutagenicity testing on o-phenylphenol. *Mutat. Res.* **54**, 227 (abstract).

Smith, R.A., Christenson, W.R., Bartels, M.J., Arnold, L.L., St John, M.K., Cano, M., Garland, E.M., Lake, S.G., Wahle, B.S., McNett, D.A. & Cohen, S.M. (1998) Urinary physiologic and chemical metabolic effects on the urothelial cytotoxicity and potential DNA adducts of *o*-phenylphenol in male rats. *Toxicol. Appl. Pharmacol.*, **150**, 402–413.

Suzuki, H., Suzuki, N., Sasaki, M. & Hiraga, K. (1985) Orthophenylphenol mutagenicity in a human cell strain. *Mutat. Res.*, **156**, 123–127.

Taniguchi, Y., Morimoto, J., Okada, K., Imai, S. & Tsubura, Y. (1981a) Toxicological study of o-phenylphenol (OPP) in mice. *J. Nara Med. Assoc.*, **32**, 425.

Taniguchi, Y., Morimoto, J., Okada, K., Imai, S. & Tsubura, Y. (1981b) Toxicological study of o-phenylphenylphenate (OPP-Na) in rats II. Acute oral toxicity in Fischer 344 DUCRJ rats. *J. Nara Med. Assoc.*, **32**, 709.

Tayama, S. & Nakagawa, Y. (1991) Sulfhydryl compounds inhibit the cyto- and genotoxicity of *o*-phenylphenol metabolites in CHO-K1 cells. *Mutat. Res.*, **259**, 1–12.

Tayama, K., Iguchi, S. & Hiraga, K. (1979) Acute oral toxicity of sodium *o*-phenylphenol (OPP-Na) in rats. *Ann. Rep. Tokyo Metr. Res. Lab. Public Health*, **30**, 57.

Tayama, K., Iguchi, S. & Hiraga, K. (1980) Acute oral toxicity of *o*-phenylphenol in rats. *Ann. Rep. Tokyo Metr. Res. Lab. Public Health*, **31**, 1.

Tayama, K., Iguchi, S., Sasaki, M. & Hiraga, K. (1983) Acute oral toxicity of *o*-phenylphenol (OPP) in mice. *Ann. Rep. Tokyo Metr. Res. Lab. Public Health*, **34**, 325

Tayama, K., Iguchi, S., Sasaki, M. & Hiraga, K. (1984) Acute oral toxicity of *o*-phenylphenol (OPP) in mice. *Toxicology*, **101**, 187 (abstract).

Tayama, S., Kamiya, N. & Nakagawa, Y. (1989) Genotoxic effects of *o*-phenylphenol metabolites in CHO-K1 cells. *Mutat. Res.*, **223**, 23–33.

Tayama-Nawai, S., Yoshida, S., Nakao, T. & Hiraga, K. (1984) Induction of chromosome aberrations and sister-chromatid exchanges in CHO-K1 cells by o-phenylphenol. *Mutat. Res.*, **141**, 95–99.

Timchalk, C. (1996) ^{14}C-Orthophenylphenol: Pharmacokinetics following dermal application in male human volunteers. Unpublished report No. HET-K-001024-064 from Dow Chemical Co., Midland, Michigan, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Timchalk, C., Selim, S., Sangha, G. & Bartels, M.J. (1998) The pharmacokinetics and metabolism of $^{14}C/^{13}C$-labeled *ortho*-phenylphenol formulation following dermal application to human volunteers. *Hum. Exp. Toxicol.*, 17, 411–417.

Ushiyama, K., Nagai, F., Nakagawa, A. & Kano, I. (1992) DNA adduct formation by ophenylphenol metabolite *in vivo* and *in vitro*. *Carcinogenesis*, 13, 1469–1473.

Wahle, B.S. & Christenson, W.R. (1996) Technical grade *ortho*-phenylphenol: A combined chronic toxicity/oncogenicity testing study in the rat. Unpublished report No. 92-272-SC from Bayer Corp., Agriculture Division, Toxicology, Stilwell, Kansas, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Yamaha, T., Tanaka, A., Sato, M. & Tsuchiya, T. (1983) Excretion, distribution and metabolic fate of sodium o-phenylphenol in the rat. Unpublished report from Division of Medical Chemistry, National Institute of Hygienic Sciences, Tokyo, Japan. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Zablotny, C.L., Breslin, W.J. & Kociba, R.J. (1991) Ortho-phenylphenol (OPP): Gavage teratology study in New Zealand white rabbits. Unpublished report No. K-001024-045 from Toxicology Research Laboratory, Health & Environmental Sciences, Dow Chemical Co., Midland, Michigan, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.

Zempel, J.A. & Szabo, J.R. (1993) Ortho-phenylphenol: 21-day repeated dermal dose study of systemic toxicity in Fischer 344 rats. Unpublished report No. K-001024-056 from Health & Environmental Sciences-Texas, Lake Jackson Research Center, Dow Chemical Co., Freeport, Texas, USA. Submitted to WHO by Leng Associates, Midland, Michigan, USA.